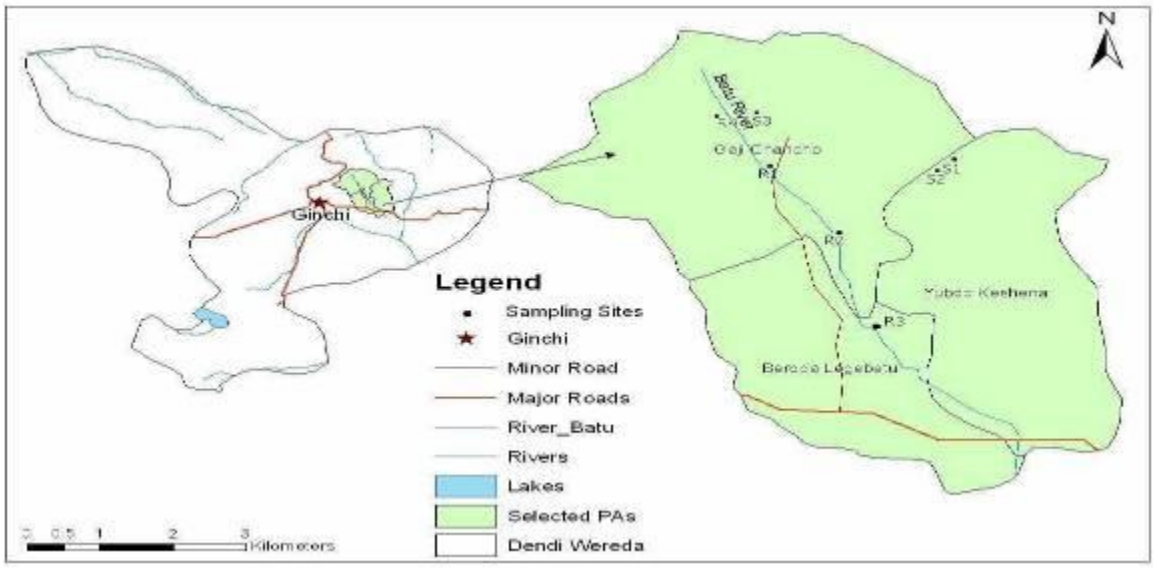
Addis Ababa University School of Graduate Studies
Department of Biology

Assessment of the contamination level of water at collection points and determination of the major sources of contaminants in the Central Highlands of Ethiopia (Yubdo-Legebatu PA).



Map showing the study area sampling points in Yubdo-Legebatu PA.

A Thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Sciences in Biology (Applied Microbiology stream)

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DEDICATION This work is dedicated to my father, Ato Million Tadesse, my mother w/o Aselefech Beyne and my sisters and brothers who selflessly dedicated their whole life to the educational betterment of their children.

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Acronyms

ANOVA	Analysis of Variance
APHA	American Public Health Association
CFU	Colony Forming Units
Cfu/g	Colony forming unites per gram
Cm	Centimeter
CPW	Challenge Program on Water and Food
EC	*Entrococcus*
E.coli	*Escherichia coli*
FC/TTC	Fecal/Thermotolerant coliform
FDRE, MoWR	Federal Democratic Republic of Ethiopia, Ministry of Water Resources
FDRE, MoH	Federal Democratic Republic of Ethiopia, Ministry of Health
FS	Fecal streptococcus
G	Gram
GIS	Geographic Information System
GPS	Global Positioning Systems
H	Hour
ILRI	International Livestock Research Institute
IWMI	International Water Management Institute
IWSC	International Water and Sanitation Center
Km	Kilometer
LES	Lewis Experimental Station
MDG	Millennium Developments Goal
M Entrococcus	Membrane Entrococcus
MFC	Membrane Fecal Coliform
Ml	Milliliter

Mm	Milimeter
μm	Micrometer
NaCl	Sodium Chloride
NTU	Nephelometers Turbidity Units
PA	Peasant Association
TC	Total coliform
WHO	World Health Organization
YLPA	Yubdo-Legebatu Peasant Association

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Abstract

Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells. Studies have confirmed that water related diseases not only remain a leading cause of morbidity and mortality worldwide but that the spectrum of disease is expanding and the incidence of many water related microbial diseases are increasing. Thus, this study was conducted to assess contamination level of water and determine the major sources of contaminants at collection points.

Three sites of three springs and four sites of a river water from Yubdo-Legebatu PA were selected for this study. Representative samples from the water bodies and livestock were collected and analyzed at AAU, Department of Biology, Applied Microbiology Laboratory. Level of contamination was determined on the bases of total coliform, fecal coliform and fecal streptococcus from the water and livestock fecal samples following the membrane filtration method. Major sources of contaminants were investigated by using the ratio of fecal coliform to fecal streptococcus for water samples.

Water analysis demonstrated that all water sources in the study area were contaminated with total coliforms. Except the sample from the undisturbed river site that had contamination 91.7%, all the others had 100% contamination with total coliforms. Out of the samples studied, 100% of spring site 1, spring site 2, spring site 3, river site 1 and river site 3, 83.3% of river site 2 and 91.7% of undisturbed river sites had unacceptable levels of total coliforms. Likewise, all water sources were 100% contaminated with fecal coliforms, except that of river site 2 and undisturbed river site which had 91.7% and 83.3% of contamination level, respectively. Out of the samples considered, 100% of

the samples from spring site 1, spring site 2, spring site 3, river site 1 and river site 2, 91.7% of river site 3 and 83.3% of undisturbed river site samples were above the limits. Analysis fecal streptococci revealed that 100% of the samples from spring site 1, spring site 2 and river site 2, 91.7% of spring site 3, 75 % river site 1 and 58.3% of undisturbed river site samples were contaminated with this bacterium. Out of the samples observed for fecal streptococci, 91.7% of spring site 1 and spring site 3, 66.8% of river site 1 and river site 3, 83.3% of spring site 2 and river site 2 and 58.3% of undisturbed river sites had unacceptable level. There was a significant difference in the average counts of TC, FC and FS among all water sites. Average concentrations of TC, FC and FS in spring site 3 were significantly higher than all other water sample sites.

Fecal coliform - fecal streptococci ratios in all water sources in this study showed that 45.0% indicated enteric contamination from human wastes and 55.0% was from domestic animal wastes.

The highest median and maximum concentrations of total coliforms in the livestock feces were 3.25 $x10^7$ cfu/g and 4.3 $x10^7$ cfu/g, from goat fecal samples at river site 2. The highest median and maximum counts of fecal coliforms were 2.05 $x10^7$ cfu/g and 2.4 $x10^7$ cfu/g, from sheep fecal samples at spring site 2. The highest median and maximum concentrations of fecal streptococcus were 1.6 $x10^7$ cfu/g and 3 $x10^7$ cfu/g, from cattle fecal samples at river site 1.

High concentration of bacterial indicators in all water sources of this study area suggested the presence of pathogenic organisms which constitute a threat to anyone consuming or in contact with these waters. The potential source of enteric organism's contamination of these water sources could be mainly explained by the predominance of open area defecation, absence of fencing of watering points that could protect the entrance of animals and drawing water with unclean cups. Therefore, protection of water sources accompanied by sanitation and hygiene promotion programs can improve the hygiene quality of rural water sources, where disinfection is not feasible.

1. Introduction

1.1. Background

Access to safe water is a fundamental human need and, therefore, a basic human right. Contaminated water jeopardizes both the physical and social health of all peoples. According to WHO, more than 80% of diseases in the world are attributed to unsafe drinking water or to inadequate sanitation practices (WHO, 2003a). Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells (WHO, 2000). In Ethiopia drinking water coverage was less than or equal to 21% for the rural, 84% for the urban and 30% for the country level. The per capita per day water consumption ranged from 3 to 20 liters with median of 8.5 liters (Abera and Mohamed, 2005).

Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide but that the spectrum of diseases is expanding and the incidence of many water-related microbial disease is increasing (WHO, 2003a). The diversity and severity of water borne diseases is greatest in tropical environments. Since most countries in tropical climates are under-developed, with poor medical services, and large populations that are under-nourished and ill-housed, water- borne diseases may have a much greater effect on public health in the tropics than in temperate areas (Jesuis and Terry, 1987). Diarrhea remains a major killer in children and it is estimated that 80% of all illness in developing countries is related to water and sanitation; and that 15% of all child deaths under the age of 5 years in developing countries results from diarrheal diseases (WHO, 2003a; 2004b).

In Ethiopia, three-fourth of the health problems of children is communicable diseases arising from the environment, especially water and sanitation (IWSC, 1989). Forty six percent of the mortality rate in children less than five years is due to diarrhea in which water-related diseases occupy a high proportion. The Ministry of Health of Ethiopia estimated that 6000 children die each day from diarrhea and dehydration (FDRE, MoH, 1997).

In rural areas and villages of Ethiopia, water for human consumption, drinking, washing (bathing, laundry), for preparation of food etc, is obtained from rivers, streams, shallow wells, springs, lakes, ponds, and rainfall. Unless water is made safe or treated for human consumption, it may be

hazardous to health and transmit diseases. The main contaminants of these water sources are human excreta, animal waste and effluent because of open field defecation practices. Thus, the majority of rural communities use water from contaminated or doubtful sources, which expose the people to various water-borne diseases (FDRE, MoWR, 2004).

Detection, differentiation and enumeration of Entrobacteriaceae are of primary importance in the microbiological quality control of water. Indicator bacteria are used to evaluate the potability of drinking water because it would be impossible to accurately enumerate all pathogenic organisms that are transmitted by water (Paccker *et al*., 1995). The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been of paramount importance in protecting public health. The principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality has been the foundation upon which protection of public health from water-borne diseases has been developed (Barrell *et al*., 2002). The presence of any coliform organism in drinking water is used as an indicator of fecal contamination since they are the most sensitive indicator bacteria for demonstrating excremental contamination (Paccker *et al*., 1995).

Thermotolerantcoliforms are the group of coliform organisms; their use in assessing water quality is considered acceptable for routine purposes (WHO, 2003b). The group contains mainly type 1 *Escherichia coli* (*E.coli*) at about 95%, which are almost exclusively derived from human and animal feces and many other bacterial species that have an environmental source (example *Citrobacter* or *Klebsiella* species). Thermotolerant coliforms other than *E.coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils (FDRE, MoH, 2006).

Fecal streptococci are also used as indicators of drinking water microbiological quality. It has repeatedly been shown that these bacteria have a stronger relationship to diarrheal disease even than *E.coli* and a closer relationship to bacterial indicators of known human fecal origin (FDRE, MoH, 2006).

Bacteriological techniques employed to distinguish between human and animal fecal pollution are a valuable tool in water pollution control programs, because they are useful in tracing the source of pollution of drinking water supplies, and they can help in assessing the overall adequacy of protection rendered to small rural water supplies (Mara and Oragui,1985). Fresh addition of human fecal material can be distinguished from additions of animal feces in environmental waters by the ratio of fecal coliforms to fecal streptococci (FC/FS).

All types of water sources may be subjected to contamination by agricultural activities. Free ranging animals may excrete feces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of fecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs (Barrell *et al*., 2002).

Management of fecal contamination of water would be improved if its sources could be accurately identified through water analysis. Human feces are generally perceived as constituting a greater human health risk than animal feces, but reliable epidemiological evidence is lacking. United States water-borne disease data suggest that human specific enteric viruses account for over half of the documented outbreaks of diarrhea. Irrespective of the relative risks, the ability to identify sources would assist in overall management of microbial water quality (Sinton *et al.*, 1998).

The mapping of water resources in the Yubdo-Legebatu Peasant Association in the Dendi district in Central Ethiopia showed that the community had access to 28 water sources including rivers and springs distributed unevenly across different land types. Most of these sources were found unsuitable for human consumption, based on visual inspection and preliminary analyses (unpublished data, ILRI). This PA was selected for a test at household level of home water treatment by sand filtration pots (Ephrem, 2007) that previously had shown a treatment efficacy of some 90 % (unpublished data, ILRI). This percentage is fine where water is of rather good quality to start with, but where it is very polluted, the 10% pollution left would make it unfit for human consumption. Probably a combination of water source protection and home water treatment together could provide people in Yubdo-Legebatu with safe drinking water.

WHO bacteriological guidelines WHO (2004a) and Federal Democratic Republic of Ethiopia, Ministry of Water Resources (2002) for drinking water recommend zero total coliforms and fecal streptococci/100 ml of water. Therefore, this study evaluates three bacterial indicators of drinking water quality (total coliform, fecal coliform/thermotolerant and *Entrococcus*/fecal *Streptococcus*) from different water sources and livestock fecal samples and determines the major sources of contamination between human and livestock and among different livestock species at Yubdo-Legebatu peasant association.

1.2. Objectives of the study.

1.2.1. General Objective

To assess the contamination levels of water at collection points and determine the major sources of contaminants in west Shoa, Dendi district,Yubdo-Legebatu PA in the Central Highlands of Ethiopia.

1.2.2 Specific Objectives

1. To assess and compare bacterial contamination levels of water at main water collection points at the river and springs.
2. To assess the bacterial concentration of indicator bacteria in livestock feces near by water collection points.
3. To determine the major sources of water contaminants at different water collection points.
4. To generate baseline information for further study.

2. Literature Review

2.1. Water and Sanitation

Water and sanitation are about much more than health (Cairncross *et al*., 2003). Access to safe drinking water is important as a health and development issue at national, regional and local levels. In some regions, it has been shown that investments in water supply and sanitation can yield a net economic benefit, since the reductions in adverse health effects and health care costs outweigh the costs of undertaking the interventions (WHO, 2004b). This is true for major water supply infrastructure investments through water treatment in the home. Experience has also shown that interventions in improving access to safe water favor the poor in particular, whether in rural or urban areas, and can be an effective part of poverty alleviation strategies (WHO, 2004b).

The WHO report indicated that globally, the percentage of people served with some form of improved water supply rose from 79% (4.1 billion) in 1990 to 82% (4.9 billion) in 2000 (WHO, 2000). The same report indicated that over the same period, the proportion of the world's population with access to excreta disposal facilities increased from 55% (2.9 billion people served) to 60% (3.6 billion). Hence, at the beginning of 2000, one-sixth (1.1 billion people) of the world's population was without access to improved water supply and two-fifths (2.4 billion people) lacked access to improved sanitation.

The objective of the United Nations Millennium Development Goals (MDGs) is to reduce persistent poverty and promote sustainable development worldwide especially in developing countries (Thompson, *et al*., 2000). Improvement of drinking water supply and sanitation is a core element of poverty reduction. The MDG target for water is to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation. WHO estimated that if these improvements were to be made in sub-Saharan Africa alone, 434,000 child deaths to diarrhoea would be averted annually (WHO, 2004b). However, some scholars suggested that the provision of water supply in developing countries may not be sufficient because of (a) high population growth, (b) conflict and political instability, and (c) low priority given to water and sanitation programs (Thompson *et al*., 2000).

Several authors have indicated that diseases caused by contaminated water are the major threat in developing countries. Esrey and his colleagues (1985) have noted that contaminated water sources can serve as a vehicle for the transmission of pathogens to human. More than one third of deaths in developing countries are caused by drinking water especially from highly contaminated sources (Ngoma, 1992).

Of the 37 major diseases in developing countries, 21 have been reported to be related to water and sanitation. In Ethiopia the World Health Organization reported that some 4.2 million people suffered from acute lack of water (WHO, 2004b).

The lack of access to safe drinking water and sanitation places a heavy burden on children who are especially vulnerable to diarrheal disease. According to the Ethiopian Ministry of Health (FDRE, MoH, 2001), diseases related to water, sanitation and hygiene problems are among the leading causes of morbidity and mortality, accounting for a large portion of the deaths of 500,000 children each year.

The study of Esrey and Habicht (1986) described a matrix of factors necessary at the community level to positively influence community health. Water quality was recognized as the foundation for any health improvement strategy, accompanied by programs for increased water quantity and sanitation. However, in the absence of changes in personal behavior and hygiene practices, the incidence of water-related diseases, for which the fecal-oral route is a major source of disease transmission, is likely to remain high in contaminated environments.

2.2. Major biological contaminants

While water is essential for life, unfortunately not all water helps man to survive. Water from contaminated sources may cause numerous diseases and untimely deaths. The fact that man needs water and cannot live without it, forces him to use it even for drinking purposes, whether clean or contaminated. As a result, people suffer from water-born diseases especially in Ethiopia. The contaminants are mainly biological pathogens. In rural villages and urban areas of Ethiopia, the main

contaminants are from human excreta, animal waste, liquid waste from factories, flourmills, garages, pesticides from different sources. Water sources contaminated with these wastes is not fit for human use, unless it is made safe or treated (FDRE, MoH, 2006).

A large variety of bacterial, viral and protozoan pathogens are capable of initiating water borne infections. The enteric bacterial pathogens include early recognized agents, such as *Salmonella* and *Shigella* species and newly recognized pathogens from fecal sources, such as *Campylobacter jejuni* and enterohaemorrhagic *Escherichia coli*. Several bacterial pathogens, such as *Legionella* species, *Aeromonas* species, *Pseudomonas aeruginosa* and *Mycobacterium avium*, have a natural reservoir in the aquatic environment and soil (Leclerc, 2003). More than 15 different groups of viruses, encompassing more than 140 distinct types, can be found in the human gut. These enteric viruses are excreted by patients as well as asymptomatic carriers and find their way into sewage. The most prevalent enteric protozoa associated with water borne diseases include *Giardia lamblia* and *Cryptosporidium parvum*. In addition, protozoa like *Cyclospora*, *Isospora* and many *Microsporidian* species are emerging as opportunistic pathogens and may have water- borne routes of transmission. Like viruses, these protozoa cannot multiply but do survive in contaminated water (Leclerc, 2003).

The most common manifestation of water-borne illness is gastrointestinal upset (nausea, vomiting, and diarrhea), and this is usually of short duration. However, in susceptible individuals such as infants, the elderly, and immuno-compromised individuals, the effects may be more severe, chronic (e.g., kidney damage) or even fatal. Other pathogens may infect the lungs, skin, eyes, central nervous system, or liver (Health Canada, 2006).

The risk to human health from water-based infections in natural watershed systems is most easily determined by assessing the concentrations of certain indicator bacteria in the water, such as fecal coliforms and streptococci. Bacteria originating from human and animal wastes also provide a clear indication of waste disposal in watershed systems and help to identify management strategies for providing clean water to the watershed residents (Monzer *et al*., 2005).

2.3. Indicator organisms and their importance

The concentration of pathogens (disease causing bacteria) in natural waters is generally very low; methods for their detection and enumeration are often complex and expensive. Alternative organisms that are consistently present in fecal material, survive reasonably well in water compared to pathogens, and are easier to detect, have therefore become widely used as fecal pollution "indicators" (Oragui and Mara, 1981; Rutkowski and Sjogren, 1987).

The most commonly used indicator organisms are the coliform bacteria, including their subset, the fecal coliforms. Coliform bacteria, thermotolerant (fecal) coliforms and *E. coli* have, for almost a century, been used as indicators of the bacterial safety of drinking-water (Leclerc *et al*., 2001).

The term "coliform organisms" refers to Gram-negative, rod-shaped bacteria capable of growing in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35-37 °C with the production of acid, gas, and aldehyde within 24-48 hours. They are also oxidase-negative and non-spore-forming (Barrell *et al*., 2002; WHO, 2003b).

Originally, total coliform bacteria were considered to be from four genera of the family Enterobacteriaceae that could all ferment lactose. These genera were *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* (Stevens *et al.*, 2003). Thermotolerant coliforms are defined as "the group of coliform organisms that are able to ferment lactose at 44-45°C"; they comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter* (WHO, 2003b).

Downstream of Victoria Falls town in Zimbabwe, sewage outfall concentrations of 7.0×10^4 fecal coliforms and 3.3×10^4 *E.coli* /100 ml were detected. In most samples the *E.coli* counts were equal to the fecal coliform counts (Sara and Sickle, 1990). All samples collected from untreated wells, streams and the river contained coliforms and fecal coliforms. Of the well water samples, 92.7% contained coliforms, as did 84.3% of the stream waters and 77.5% of the river waters (Antai, 1987).

Result of the El-Kabir river watershed in Lebanon and Syria indicated that total coliform (TC) and fecal coliform (FC) concentrations were extremely high throughout the watershed, rendering the water unfit for any human use. The primarily human origin of the pollution is supported by high ratios of FC/FS, although impacts from animal wastes were also observed. Spring water also exhibited elevated levels of bacteria, implicating surface land use and waste disposal practices upstream of the springs. Mean values of river and spring water for total coliforms, fecal coliforms and fecal streptococci per 100 ml were 38 000, 23 000, 177 040, 49 563 and 14 600, 20 000 respectively. Ratios of fecal coliform/fecal streptococci for the three sampling periods' samples were 1.4 and 1.1, 6.7, 16.7 (Monzer *et al*., 2005).

In a monitoring study in the Geum River in Korea, the lowest and highest average concentrations of total coliforms at six sampling stations were 1670 and 8510 cfu/100 ml. Where as the lowest and highest average concentrations of fecal coliforms were 170 and 4450 cfu/100 ml (Geonha *et al*., 2005). A survey of the microbiological quality of a natural spring, located in an area with little interference by human and animals, was conducted in Seoul, South Korea. Total coliforms were detected in all samples and the mean density of total coliforms was up to a maximum of 228 cfu/ml. Detectable *E. coli* are found in 78% of all samples and the mean densities of *E. coli* varied from a minimum of 0 cfu/ml to a maximum of 15 cfu/ml in all samples (Youn-Joo and Breindenbach, 2005).

In a study conducted in Gondar, Ethiopia, 75% of the samples taken from unprotected wells and springs were contaminated by fecal coliforms, especially *E.coli* (Mengesha *et al*., 2004). The authors further reported that fifty percent of the samples in both cases had a coliform count of 180/100 ml and above. No sample in either case had a coliform count of less than 10/100 ml. The least coliform count seen was 13 coliform /100 ml and on the basis of these, they concluded that the majority of the drinking water sources were either of unacceptable quality or grossly polluted (Mengesha *et al*., 2004).

Fecal streptococci are the most commonly used alternative or adjunct to coliform bacteria as fecal pollution indicators. The fecal streptococcus group consists of a number of species of the genus

Streptococcus, such as *S. faecals, S. faecium, S. avium, S. bovis*, *S. equinus,* and *S. gallinarum*. The normal habitat of the fecal *Streptococcus* is the gastrointestinal tract of warm-blooded animals. *S. faecalis* and *S. faecium* once were thought to be more human-specific than other S*treptococcus* species. Other species have been observed in human feces but less frequently. Similarly, *S. bovis, S. equinus* and *S. avium* are not exclusive to animals, although they usually occur at higher densities in animals (APHA, 1998).

Fecal streptococci have been suggested as possible indicators to help to differentiate between pollution of human and animal origin (Rutkowski and Sjogren, 1987). Fecal streptococci rarely multiply in polluted water and are more persistent than *E.coli*. High enterococci concentrations, on the other hand, are usually associated with raw sewage discharge and discharge/run-off from meat processing plants, dairy farms or feedlots and livestock range (Irvine and Pettibone, 1996; Laukova and Juris, 1997; Pinto *et al*., 1999). The *entrococcus* group is a subgroup of the fecal streptococci that includes *S. faecals*, *S. faecium*, *S. gallinarum* and *S. avium.* The entrococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at 9.6 pH and at 10^{o}C and 45^{o}C (APHA, 1998). More recent research on the relevance of fecal streptococci as indicators of pollution showed that the majority of entrococci (84%) isolated from a variety of polluted water sources were "true fecal species" (Pinto *et al*., 1999).

Enterococci can provide an indication of past pollution. Examination for enterococci also assists the interpretation of doubtful results from other tests such as the occurrence of large numbers of coliforms in the absence of *E.coli* (Standards Unit, Evaluation and Standards Laboratory, 2005).

A French study identified higher rates of illness among inhabitants of villages whose drinking water failed European directive standards than among inhabitants of villages whose drinking water satisfied the standards. The marker mostly closely associated with illness was the entrococci count, although fecal coliforms were also independently associated with illness (Barrell *et al*., 2000).

There are two possible ways of using fecal streptococci as biological signatures of different fecal sources: the first involves comparing their concentrations to those of fecal coliforms; the second

entails identification of the constituent species in the different fecal sources and receiving waters. Reports of streptococcal survival in the environment compared to other indicators are often inconclusive or contradictory. However, most studies have shown that fecal streptococci outlive coliforms and fecal coliforms in effluents and aquatic environments (Sinton *et al*., 1993).

Streptococcal concentrations in human feces (in the order of 10^6/g) are generally less than fecal coliforms (Sinton and Donnison, 1994). Fecal coliforms concentrations per gram of human feces estimated $1.3x10^7$ (Geldreich, 1978).

It has been reported that FC/FS ratios > 4 are associated with human waste, between 0.1 and 4.0 with domesticated animals like cattle, while those < 0.1 are thought to be indicative of wild animals' wastes (Coyne and Howell, 1994). But, due to the differential survival rates and other factors, this ratio is not reliable if the fecal contamination is not fresh, or if the contamination of fecal streptococci is less than 100 cfu/100 ml (APHA, 1998). This was demonstrated in a recent study in South Africa where the addition of human fecal material into an agriculturally impacted river showed a rise in the FC/FS ratios, but further downstream the ratio fell to levels that would not indicate the presence of domestic sewage (Jagals and Grabow, 1996).

2.4. Sources of pollution

The primary source of microbial pollution in agricultural watersheds is fecal matter from livestock production. The microbial loading potential from point sources, such as storage facilities and feedlots, and from non-point sources, such as grazed pastures and rangelands, is substantial (Jamieson *et al*., 2004). Walker *et al*. (1990) stated that source areas can be divided into four categories: (i) areas where manure is surface applied, (ii) areas where manure is incorporated into the soil, (iii) areas where manure is directly deposited by livestock, and (iv) non-manured areas. Although a variety of protozoa and bacteria can be shed by livestock and transmitted to humans through water, animal agriculture is likely responsible for a percentage of many of the pathogens we find in surface water, but whether that percentage is 5%, 50% or 95% compared to non-agricultural sources such as humans or wildlife is unknown at this time for most watersheds (Edward, 1995).

In animal feces, the fecal streptococci generally outnumber fecal coliforms, although the overall concentrations appear to differ markedly between species. For example, sheep feces contain approximately 3.8×10^7 fecal streptococci /g compared to 1.6×10^7 fecal coliforms/g. Cow feces contain 1.3×10^6 fecal streptococci/g and 2.3×10^5 fecal coliforms/g. In contrast, streptococcal concentrations in human feces which are typically around 3.0×10^6 /g are generally less than those for fecal coliforms, which are typically around 1.3×10^7 /g (Mara, 1974). Hence the ratio between fecal coliform and streptococcal bacteria in water can help to identify the main source of pollution.

Cattle have been shown to produce 5.4 billion fecal coliform and 31 billion fecal *Streptococcus* bacteria in their feces per day. Since cattle spend a significant portion of their time in or near streams, lakes, and wetland areas and average 12 defecations per day, they can contribute significant numbers of these organisms to surface waters (Howard *et al*., 1983).

Runoff from a cow-calf pasture in eastern Nebraska was monitored for total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) during 1976, 1977, and 1978 (Doran and Linn, 1979). Bacteriological counts in runoff from both grazed and ungrazed areas generally exceeded recommended water quality standards. The FC group was the best indicator group of the impact of grazing. Rainfall runoff from the grazed area contained 5 to 10 times more FC than runoff from the fenced, ungrazed area. There was little difference in TC counts between the two areas, but FS counts were higher in runoff from the ungrazed area and reflected the contributions from wildlife. The FC/FS ratio in pasture runoff was useful in identifying the relative contributions of cattle and wildlife. Ratios below 0.05 were indicative of wildlife sources and ratios above 0.1 were characteristic of grazing cattle. Occasions when the FC/FS ratio of diluted cattle waste exceeded one resulted from differential after growth and die-off between FC and FS (Doran and Linn, 1979).

A fecal analysis survey undertaken to quantify animal inputs of pathogenic indicator microorganisms in the temperate watersheds of Sydney, Australia indicated that pathogen and fecal indicator concentration were generally higher in domestic animal feces than wildlife feces (Cox *et al*., 2005).

2.5. Environmental factors influencing pathogens

Once a pathogen leaves the host environment, it must adjust to external conditions that are different and stressful. The length of survival is extended from days to possibly months in relatively protected (lack of predators, toxins, sunlight, and cooler), moist, nutritive environments such as sediments and deep soils (> 40cm). It is assumed that gastrointestinal pathogens in fecal matter will die at the same rate as the indicator bacteria; therefore, high concentrations of fecal indicator bacteria indicate an increased likelihood of pathogens being present (Cabelli *et al*., 1982).

The survival of most pathogens is highly variable depending upon the receiving water, particularly turbidity, temperature, oxygen levels, presence of nutrients and pesticides, pH, organic matter, and solar radiation (Moore *et al*., 1988). Temperature, pH, moisture, nutrient supply and solar radiation seem to have the greatest effect on enteric bacterial survival. Lower temperatures appear to increase survival time as noted by (Kibbey *et al*., 1978). This investigator also indicated that temperature extremes seem to be most disruptive to bacterial survival. Most bacterial pathogens are sensitive to temperatures exceeding 60 degrees Celsius. Bacterial pathogens can produce resistant endospores or thick-walled cells and only be killed by high temperatures in excess of 100 degrees Celsius. The normal pH range for most water bodies is close to 7 (neutral) and would not affect bacteria survival. Only at extreme pH (< 4.5 or > 8.2) can cell die-off be expected (James, 1999).

Turbidity in addition to temperature and pH can also influence the microbiological quality of drinking water. Therefore, these are recommended in water monitoring programs as they may influence disinfection efficiency and microbial survival (FDRE, MoH, 2006). Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity caused by high levels of organic matter can protect microorganisms from the effects of disinfection. It even can stimulate bacterial growth. Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. The maximum contaminant level turbidity for public potable water is 1 NTU (Environmental Monitoring and assessments, 2006).

3. Materials and Methods

3.1. Description of the study area.

The study was conducted in the Yubdo-Legebatu Peasant Association (YLPA). It is located at about 80 km west of Addis Ababa in the Dendi district of Oromiya Region. The PA is located at about 20 km from the district town Ginchi. The study area has uneven topography with upland, midslopes and bottomlands. It receives mean annual rainfall ranging from 800-1172 mm and has an average temperature between 9.3°C and 23.8°C. The altitude of the area ranges between 1600 and 3268 meter above sea level. The total population in YLPA is 5614 and the number of households in upland and bottomland of YLPA is 796 (unpublished data, Dendi Woreda Bureau of Agriculture). The mapping of water resources in Yubdo-Legebatu showed that the community had access to 28 water sources including rivers and springs distributed unevenly across different land types. Most of these sources were found unsuitable for human consumption (unpublished data, ILRI). The watersheds have mixed land use, with significant agricultural activities in rural residential areas.

3.2. Set-up of the study

Water samples were collected from three unprotected springs in the upland part of the settlement and Legebatu River representing the main watering points where the human and livestock population depend on for their daily water supply for drinking and other domestic purposes. During sampling, three sites of springs and four points along the river were chosen. A site in the upper river, which is not used by people and livestock, was used as a control to assess and compare contamination levels with the most utilized downstream sites of the river and springs. The Legebatu River flows down from the upper river site through river site 1, 2 and 3 across the villages and cultivated land. Selected river sites were 1-1.5 km away from each other along the water course (Fig.1). In all cases composite samples were used for analysis. Samples were collected aseptically and kept in an ice-box during transport.

A membrane filtration technique was used to detect and enumerate total coliform, fecal coliform, *Entrococcus/*fecal *Streptococcus* from both water and livestock fecal samples following the APHA (1998) procedure. The membrane filter technique that involves direct plating for detection and

estimation of coliform and fecal *Streptococcus* densities, is one of the best techniques currently available (APHA,1998).

A questionnaire was prepared and general information was collected from the community to increase the quality and compatibility of information on improved drinking water sources and sanitation. A handheld GPS was used to collect GIS data to locate sampling points on the topographic map of the study areas.

3.3. Samples and sampling points

3.3.1. Water

Six rounds of triplicate water samples were taken from each site: three springs and four sites along Legebatu River between November 2006 and February 2007(Figures 1, 2, 3). Samples were collected following APHA (1998) sampling procedure. In each round of sampling, one sample was taken at the center and the other two samples from the two edges of each site. The water samples were handled aseptically in sterile glass bottles, labeled and kept in an ice-box during transportation to the Applied Microbiology Laboratory at the Department of Biology in Addis Ababa University.

The three water samples from each site were mixed and two composite samples were taken and analyzed in the laboratory. Twelve water samples from each site were thus analyzed in six rounds. Totally 84 water samples, 36 from three springs and 48 from the four sites of the river were analyzed.

Water temperature was measured at each water sampling site prior to sample collection. pH and turbidity were measured after samples arrived at the laboratory. The pH meter was rinsed with distilled water between each sampling. Samples were returned to the laboratory eight hours after collection and processed immediately.

3.3.2. Feces

Random livestock (cattle, sheep, goat, donkey and horse) fresh fecal samples were collected from the surface with sterile spatulas and transferred to sterile bottles. Two different composite samples were made. A composite sample was prepared from three different fecal samples, each from the

same species of livestock at all water sampling points except the river control site. All bottles were labeled and kept in an ice-box until arrival at the laboratory.

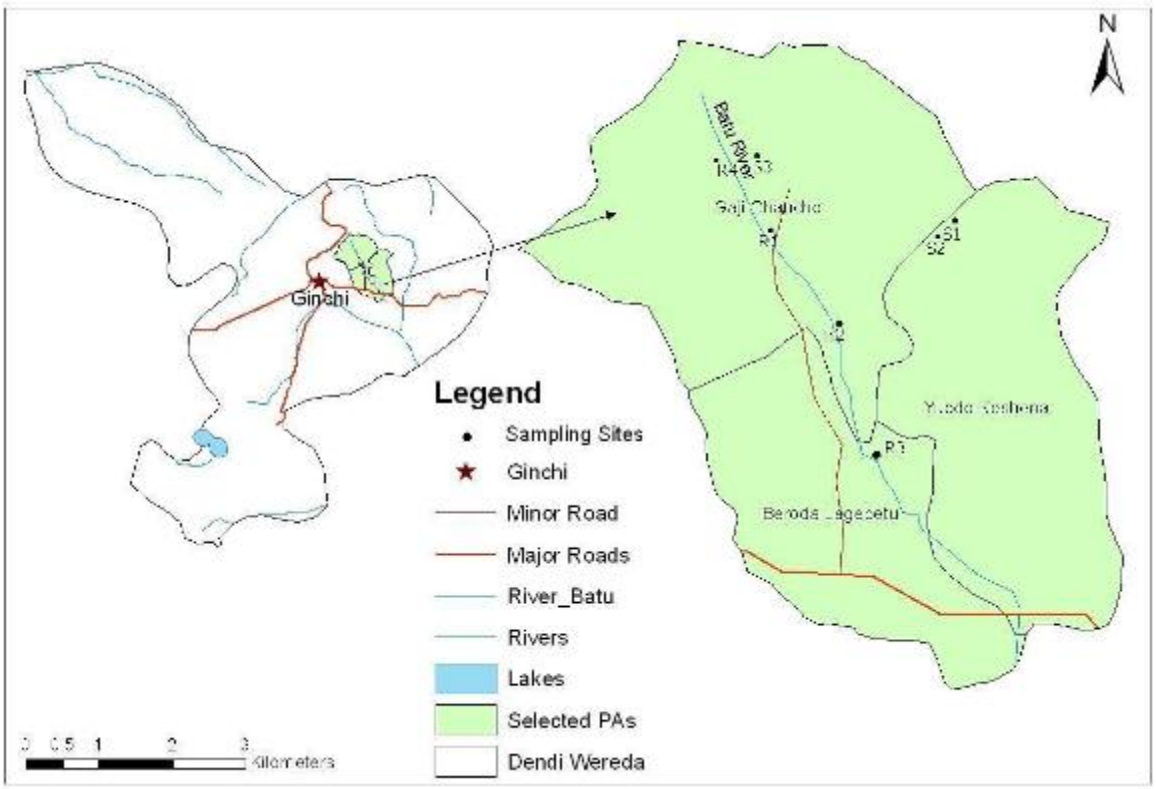


Figure 1: Map showing sampling points in Yubdo-Legebatu PA**.**

S1 – Spring 1	R1 – River site 1
S2 – Spring 2	R2 – River site 2
S3 – Spring 3	R3 – River site 3
	R4 – Upper river site

Spring 1

Spring 2

Spring 3

Spring 3

Figure 2: Figure of sampling points: springs

River site 1

River site 2

River site 3

Upper river site

Figure 3: Figure of sampling points: river

3.4. Analyses of samples

3.4.1. Water samples

Composite samples were used to improve the precision (lower the variance) of the estimated average contaminant concentrations. In the laboratory, the three samples from each site were mixed into one and two replicate samples (10 ml each) were subjected for membrane filter analysis of total coliforms(TC), fecal coliforms(TTC) and *Entroccoccus/* fecal *Streptococcus*(FS*)*.

The composite samples were mixed thoroughly by shaking and filtered under laboratory hood, using WagTech Membrane Filtration apparatus and membranes, pore size 0.45µm, 47mm diameter, sterile and gridded. The membranes were then transferred aseptically to m-Endo Agar LES, m-FC agar with rosolic acid and mEntrococcus agar media in glass Petri dishes for TC, TTC, and FS respectively.

Prepared culture dishes were inverted and incubated for 24h at 35°C and 44.5°C and for 48h at 35°C for TC, TTC, and FS, respectively. Upon completion of the incubation period, typical TC colonies (a pink to dark red color with sheen), blue colored for TTC and dark red colonies for FS on the surface of membrane filter were counted using a low power binocular wide field dissecting microscope, with a cool white fluorescent light source for optimal viewing sheen. Buffer rinse water was prepared according to APHA (1998) and used to rinse the funnel between each site sample filtration.

Verification tests were done by transferring growth from each colony and placing it in lauryl tryptose broth at 35±0.5°C for 48h. Gas formed in lauryl tryptose broth within 48h verified the colonies as TC. Inclusion of EC broth for 44.5±0.2°C incubation verified the colonies as TTC/FC. Further identification was done by examining the colonies under an epifluorescence microscope attached to a digital camera.

For isolation of *Entrococcus* and fecal *Streptococcus,* typical colonies from mEntrococcus agar membrane were streaked on the surface of brain-heart infusion agar plate and incubated at 35°C for 24h. A loopful growth from a well-isolated colony on brain-heart infusion agar was transferred to brain-heart infusion broth tube and to each of two clean glass slides. The brain-heart infusion broth

was incubated at 35°C for 24h. A freshly prepared 3% hydrogen peroxide was dropped to the smear on a slide and detected.

A loopful of growth from the brain-heart infusion broth was transferred to bile esculin agar (was prepared according to the direction of APHA, 1998) and incubated at 35°C for 48h, and brain-heart infusion broth with 6.5%NaCl and incubated at 35°C for 48h. Typical colonies from mEntrococcus agar membrane were streaked, prepared for epiflourescence microscope and seen as diploid and small chain coccid shape cells, which is a typical characteristic of the indicator group (e*ntrococcus/streptococcus*).

3.4.2. Fecal samples

To get appropriate numbers of colonies on Petri plates, one gram of composite fecal samples of each species was diluted in a buffer solution to 10^{-6} (1/1,000,000) of the original samples and 1 ml sample volume was filtered and analyzed for the concentration of total coliform, fecal coliform, and fecal *Streptococcus*. A total of 108 fecal samples were analyzed following the same procedure as used for the water analysis.

3.5. Statistical analysis

Results of water analyses were compared against standards set by WHO (2004a) and Federal Democratic Republic of Ethiopia, Ministry of Water Resource (FDRE, MoWR, 2002). Analysis of variance (ANOVA) at 5% level of significance was used to compare the quality of water among all sites. The results were analyzed using statistical software SAS version 8.0 and SPSS version 13.0.

4. **Results**

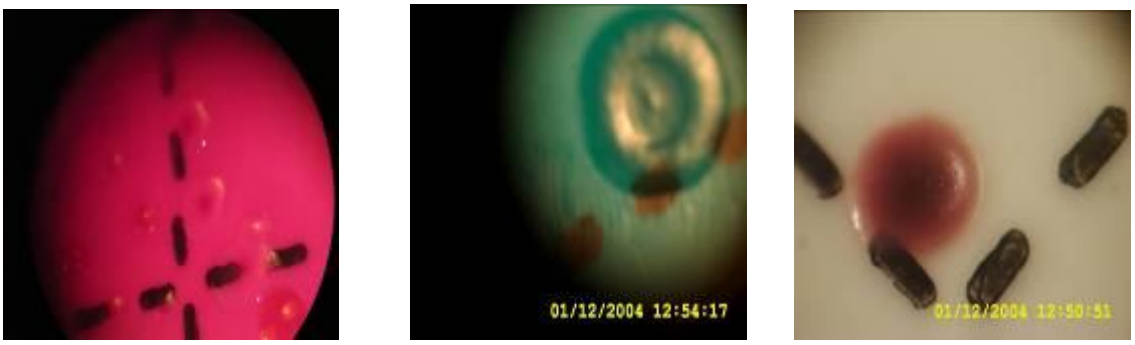
4.1. Bacteriological analysis of water samples

4.1.1. Indicator bacteria detection in the water samples.

Bacteriological analysis of samples from the three springs and four river sites at Yubdo-Legebatu PA showed that all samples except those from the upper river site were positive for total coliforms in six rounds of sampling (Figures 4, 5). Indicator bacteria were often encountered in all samples from water sources of the study area. Less frequent occurrence of indicators was observed at samples from the upper river site (Table 1).

Table 1. Bacteriological analysis of water sample from unprotected springs and a river in Yubdo-Legebatu PA between November 2006 and February 2007.

Type of organisms	Sampled water sites													
	Spring site 1 (n=12)		Spring site 2 (n=12)		Spring site 3 (n=12)		River site 1 (n=12)		River site 2 (n=12)		River site 3 (n=12)		Upper river site (n=12)	
	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%
TC	12	100	12	100	12	100	12	100	12	100	12	100	11	91.7
TTC	12	100	12	100	12	100	12	100	11	91.7	12	100	10	83.3
FS	12	100	12	100	11	91.7	9	75	12	100	11	91.7	7	58.3



(a) (b) (c)

Figure 4: Figures of TC (a), TTC (b) and FS (c) colonies in water sources of the study sites (magnified by 15x binocular wide field dissecting microscope).

(a) (b) (c)

Figure 5: Epiflourscent microscope images of TC (a), TTC (b) and FS (c) in water sources of the study sites.

4.1.2. Bacterial indicator concentrations in the water samples.

The highest average bacteriological counts were observed at spring site 3: 1447.0 total coliforms, 741.7 fecal coliforms and 411.7 fecal streptococci. The lowest mean counts 198.3 total coliforms, 75.8 fecal coliforms and 30.8 fecal streptococci were found at the upper river site (Table 2).

Table 2. Mean bacteriological counts, fecal coliform (fc)-fecal *Streptococcus* (fs) ratio, temperature, turbidity and pH values of water sources in Yubdo-Legebatu between November 2006 and February 2007 (n = 12 for each site).

Sites	Total coliform	Fecal coliform	Fecal *Streptococcus*	FC/FS	Temperature	Turbidity	pH

Spring 1	940.0±480.1	405.8±147.5	120.8±79.9	3.4	17.65±0.6	7.67±6.7	7.62±0.5
Spring 2	950.8±675.3	365.0±290.7	100.8±166.5	3.6	17.80±0.5	16.33±0.8	7.79±0.7
Spring 3	1447.0±516.5	741.7±449.7	411.7±481.5	1.8	18.00±0.4	13.83±2.8	7.26±0.6
River site1	884.2±730.3	502.5±412.6	90.00±133.9	5.6	17.85±0.4	7.17±2.0	8.10±0.4
River site 2	812.2±566.9	419.2±462.2	168.3±212.4	2.5	18.0±0.3	5.33±2.3	8.13±0.2
River site 3	831.8±627.8	495.8±523.7	94.2±168.9	5.3	18.42±0.5	5.67±2.8	8.08±0.2
Upper river site	198.3±162.3	75.8±83.6	30.8±48.1	2.5	16.82±0.7	7.17±3.3	8.0±0.2

4.1.3. Fecal coliform/fecal *Streptococcus* ratios

Following the concept of this ratio is not reliable if the contamination of fecal streptococci is less than 100 cfu/100 ml (APHA, 1998). Hence, FC/FS ratios were computed only for sites with mean FS counts ≥100cfu/100 ml water samples. To differentiate the sources of contamination the method of (Coyne and Howell, 1994) was used.

FC/FS< 0.1 - the ratio less than 0.1 for wild life wastes.

0.1≤ FC/FS ≤ 4 - the ratio between 0.1 and 4.0 for domestic animal waste.

FC/FS >4 - the ratio greater than 4 for human wastes

With this definition among the considered FC/FS ratios in all spring sites and river site 2 pollution could be derived from livestock wastes. While, results of FC/FS ratios in the remaining sites of the river were not considered due to the mean FS counts were less than 100cfu/100 ml water samples.

4.1.4. Degree of bacterial pollution in water samples.

The degree of bacterial pollution in the water samples was very high. The bacteriological counts in most sites were in the dangerous range of pollution for drinking (101-1000 cfu/100 ml). None of the water sources were found to be safe for drinking. Moreover, most of water samples taken from spring site 3 had very high pollution levels categorized under dangerous and very dangerous. While samples from the upper river site had lower pollution levels, none of the other samples could be categorized under the very dangerous degree of pollution (Table 3).

Table 3. Degree of bacterial pollution in water sources of Yubdo-Legebatu PA as percentage of samples in each category.

Sites	Pollution classes (bacterial cfu/100ml)				
	0	1-10	11-100	101-1000	>1000
Spring 1					
TC	0	0	0	83.3	16.7
TTC	0	0	0	100	0
FS	0	8.3	25.1	66.6	0
Spring 2					
TC	0	0	8.3	49.9	41.8
TTC	0	0	16.7	83.3	0
FS	0	16.7	58.4	24.9	0
Spring 3					
TC	0	0	0	20	80
TTC	0	0	0	83.3	16.7
FS	8.3	0	41.7	41.7	8.3
River site 1					
TC	0	0	8.3	49.9	41.8
TTC	0	0	16.6	75.1	8.3
FS	24.9	8.3	41.9	24.9	0
River site 2					
TC	0	16.7	49.9	33.4	0
TTC	0	0	16.7	58.4	24.9
FS	0	16.7	49.9	33.4	0
River site 3					
TC	0	0	0	72.7	27.3
TTC	0	8.3	0	74.8	16.9
FS	8.3	24.9	49.9	16.9	0
Upper river site					
TC	8.3	0	24.9	66.8	0
TTC	16.7	0	66.6	16.7	0
FS	41.7	8.3	41.7	8.3	0

0 – Safe water, 1-10 – reasonable quality, 11-100 – polluted, 101-1000 – dangerous,

>1000 – very dangerous (WHO, 2004a; FDRE, MoWR, 2002).

4.1.5. Analysis of variance among TC, TTC and FS in all water samples.

Analysis of variance was used to test whether the average counts of TC, TTC and FS were the same or not in all water sources at 5% level of significance. The results of ANOVA for differences among sites for TC, TTC and FS are summarized in Table 4, Table 5 and Table 6 in that order for each parameter. The result of ANOVA showed that there was a significant difference ($p< 0.01$) in the average counts of TC among all water sites (Table 4). Similarly, average counts of TTC and FS significantly differed among sites (Table 5 and 6).

Table 4. ANOVA of TC among all sites of springs and river.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sites	6	3688.65	614.77	20.37	< 0.01
date	5	1327.06	265.41	8.79	< 0.01
replication	5	0.002	0.002	0.00	0.99
sites*date	30	4875.87	162.53	5.38	< 0.01

Table 5. ANOVA of TTC among all sites of springs and river.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sites	6	2239.32	373.22	18.39	< 0.01
date	5	589.34	117.87	5.81	0.0004
replication	5	54.56	54.56	2.69	0.11
sites*date	30	3950.94	131.69	6.49	< 0.01

Table 6. ANOVA of FS among all sites of springs and river.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sites	6	1135.59	189.27	8.73	< 0.01
date	5	1017.15	203.43	9.38	< 0.01
replication	5	12.91	12.91	0.60	0.49

sites*date	30	2113.28	70.44	3.25	0.0003

4.2. Trends of water contamination levels over time

Contamination levels of the indicators at each site were not consistent over the six sampling rounds. Average concentrations TC, TTC and FS in all river and spring sites (means of bacterial indicators were square root transformed) were not consistent over time. In most cases the highest average indicator bacteria concentrations in both river and springs were observed in January and February. While the lowest average counts were mostly recorded in December (figures: 6, 7, 8, 9, 10, 11 and 12).

Figure 6: Trends of baterial concentrations in water samples from river site 1 over the six sampling dates

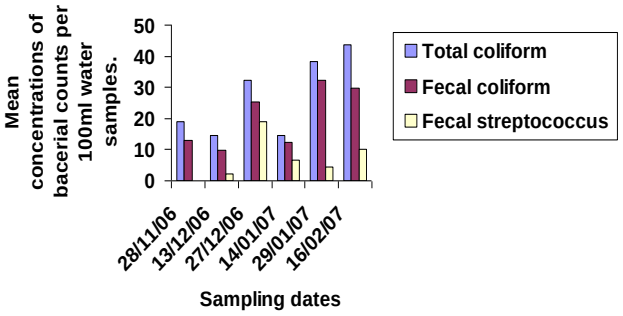


Figure 7 : Trends of bacterial concentrations over the six sampling dates in River site 2 water samples.

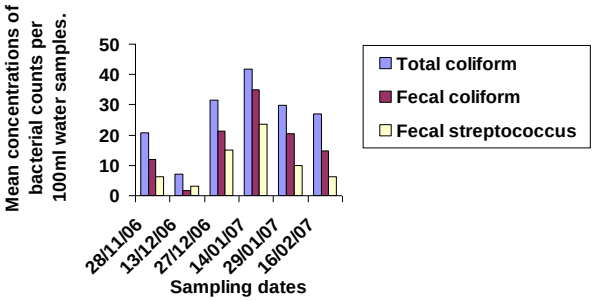


Figure 8 : Trends of bacterial concentrations in water samples from river site 3 over the six sampling dates

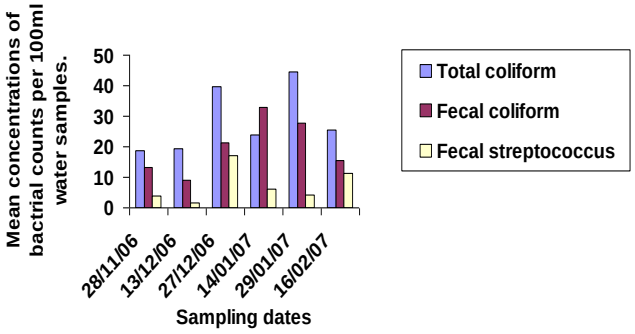


Figure 9: Trends of bacterial concentrations in water samples from upper river site over the six sampling dates.

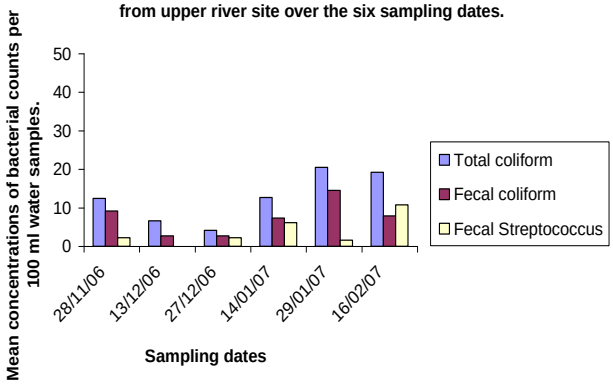


Figure 10: Trends of bacterial concentrations in water samples from spring site 1 over the six sampling dates.

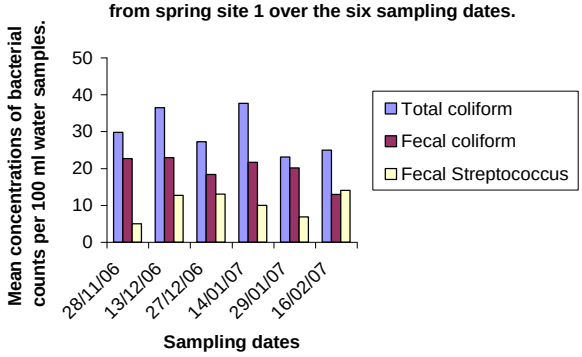


Figure 11 : Trends of bacterial concentrations in water samples from spring site 2 over the six sampling dates

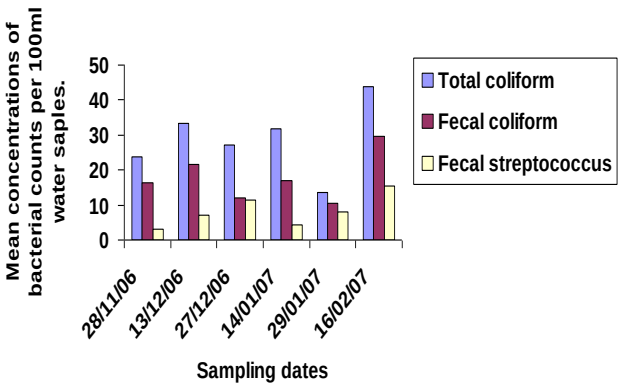
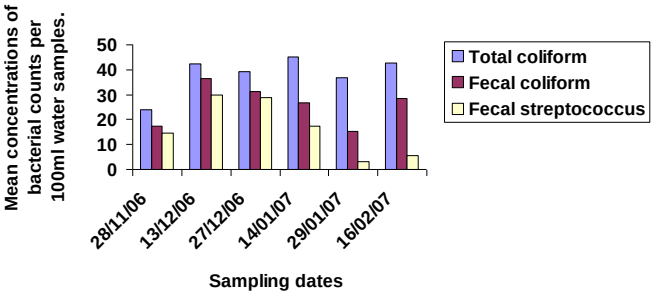


Figure 12 : Trends of bacterial concentrations in water samples from spring site 3 over the six sampling dates



4.3. Pearson's correlation between bacterial indicator counts and temperature, turbidity and pH of water sources.

The data were analyzed using Pearson's correlation to see the correlation of bacterial indicator counts with temperature, turbidity and pH of water sources (springs and river), details of which are listed in Annex 2. The result revealed that there was a highly significant positive relationship between bacterial counts and temperature ($r = 0.27$, $p < 0.01$), and between bacterial counts and turbidity ($r = 0.21$, $p = 0.0008$). In addition there was a significant negative relationship between bacterial count and pH ($r = -0.15$, $P = 0.02$) (Table 7).

Table 7. Pearson's correlation coefficients of indicator counts with temperature, turbidity and pH of water sources (grouped by spring and river).

	Bacterial counts	Temperature	Turbidity	pH
Bacterial counts	1.00 249	0.27 < 0.01 249	0.21 0.0008 249	-0.157 0.02 249

4.4. Bacteriological analysis of livestock fecal samples

Estimated average livestock herd sizes in the study area were: 3970 cattle, 1987 sheep, 1191 goats, 1191 donkeys, and 1191 horses. All types of herds were found all over the study site using nearest water sources to their village. Except donkeys and mules/horses used for transportation away from the village, which drink twice a day, most of the herds have free roaming access to drinking water.

All pooled fecal samples from livestock contained high levels of total coliforms, fecal coliforms and fecal *Streptococcus* at all sites (Table 8). The highest median and maximum concentrations of total coliforms in the livestock feces were 3.25 $x10^7$ cfu/g and 4.3 $x10^7$ cfu/g, from goat and sheep fecal samples at river site 2 and spring 2, respectively. The highest median and maximum counts of fecal coliforms were 2.05 $x10^7$ cfu/g and 2.4 $x10^7$ cfu/g, from goat and sheep fecal samples at river site 2 and spring 2, respectively. The highest median and maximum concentrations of fecal streptococci were 1.6 $x10^7$ cfu/g and 3 $x10^7$ cfu/g, from cattle fecal samples at river site 1. All fecal samples of the livestock were positive for the selected indicator bacteria (Table 8).

Table 8. Concentration of indicators per gram wet weight of livestock fecal samples in Yubdo-Legebatu, collected in March, 2007.

Sites	Livestock	Total coliform Conc. (cfu/g[wet weight])		Fecal coliform Conc. (cfu/g[wet weight])		Fecal *Streptococcus* Conc. (cfu/g[wet weight])	
		Median	Range	Median	Range	Median	Range
Spring 1	Cattle	3.5×10^6	3×10^6- 4×10^6	1.5×10^6	1×10^6-2×10^6	2.5×10^6	2×10^6-3×10^6
	Donkey	9.5×10^6	5×10^6-1.4×10^7	4.5×10^6	3×10^6-6×10^6	4×10^6	3×10^6- 5×10^6
	Horse	4×10^6	3×10^6-5×10^6	1.5×10^6	1×10^6-2×10^6	6×10^6	5×10^6-7×10^6
Spring 2	Cattle	1×10^7	6×10^6-1.4×10^7	4×10^6	3×10^6-5×10^6	3×10^6	2×10^6-4×0^6
	Donkey	1×10^7	4×10^6-1.6×10^7	5×10^6	3×10^6-7×10^6	6.5×10^6	2×10^6-$1.1\ 10^7$
	Goat	5.5×10^6	4×10^6-7×10^6	3×10^6	2×10^6-4×0^6	9.5×10^6	6×10^6-1.3×10^7
	Sheep	2.35×10^7	4×10^6-4.3×10^7	1.3×10^7	2×10^6-2.4×10^7	1×10^7	5×10^6-1.5×10^7
Spring 3	Cattle	1.3×10^7	8×10^6-1.8×10^7	5.5×10^6	3×10^6-8×10^6	1.2×10^7	3×10^6-$2.1\times\ 10^7$
	Donkey	5.5×10^6	3×10^6-8×10^6	2.5×10^6	1×10^6-$4\times\ 10^6$	4×10^6	2×10^6-6×10^6
	Horse	1.55×10^7	9×10^6-2.2×10^7	1×10^7	6×10^6-$1.4\times\ 10^7$	1.4×10^7	5×10^6-2.3×10^7
River site 1	Cattle	1.7×10^7	1.3×10^7-2.1×10^7	8×10^6	7×10^6-$9\times\ 10^6$	1.6×10^7	2×10^6-3×10^7
	Sheep	1.2×10^7	9×10^6-1.5×10^7	5×10^6	2×10^6-8×10^6	9×10^6	5×10^6-1.3×10^7
River site 2	Cattle	1.55×10^7	5×10^6-$2.6\times\ 10^7$	9.5×10^6	3×10^6-1.6×10^7	1.25×10^7	5×10^6-2.0×10^7
	Goat	3.25×10^7	2.7×10^7-3.8×10^7	2.05×10^7	1.9×10^7-2.2×10^7	7.5×10^6	3×10^6-$1.2\ \times10^7$
	Sheep	1.1×10^7	6×10^6-1.6×10^7	5.5×10^6	3×10^6-$8\ \times10^6$	4×10^6	3×10^6-5×10^6
River site 3	Cattle	6×10^6	3×10^6-9×10^6	3.5×10^6	1×10^6-$6\times\ 10^6$	3.5×10^6	2×10^6-5×10^6
	Horse	6.5×10^6	4×10^6-9×10^6	1.5×10^6	1×10^6-2×10^6	1.25×10^7	3×10^6-2.2×10^7
	Sheep	1.4×10^7	3×10^6-2.5×10^7	6×10^6	1×10^6-1.1×10^7	1.15×10^7	4×10^6-$1.9\times\ 10^7$

4.5. Results of questionnaire survey

The questionnaire survey was done immediately before starting the laboratory water analyses. It lasted two days on October 21 and 22, 2006. Five randomly selected households from the villages that use water from each of the sampling sites (except the undisturbed river site) were interviewed, the total number of households interviewed being 30.

All interviewees across the river responded that they use the Legebatu River during both dry and rainy seasons for drinking and domestic consumption without any water treatments. Similarly, all selected households away from the river responded that they use only springs in all seasons without any water treatment.

The data from the respondents (Table 9) indicated that in all cases they do not use latrines rather they use open areas nearby water sources. Similarly, all thirty households responded that they practice agricultural activities and graze their livestock nearby water sources, but do not fence watering points to prevent the entrance of animals. The selected households utilize livestock dung, in addition to other purposes, for manuring crop fields, which are nearby water sources. Though livestock type and herd size vary over the selected households in general cattle population ranged from a herd size of 2-8 and sheep were owned by some residents in numbers of 1- 4 per household. Comparably, goats, donkeys and horses herd sizes were small, with individuals' households having only 1 or 2.

Table 9. Summary of major sanitary risk factors as identified from house hold level questionnaires (n=30) in Yubdo-Legebatu, in October, 2007.

Sanitary risk factors	% of respondents			
	Upland (n=15)		Bottomland (n=15)	
	Yes	No	Yes	No
Practice of using toilet	0	100	0	100
Fence watering points	0	100	0	100
Graze livestock nearby water sources	100	0	100	0
Practice of any water treatment	0	100	0	100
Practice of agricultural activities nearby water sources	43.3	56.7	73.3	26.7
cattle owning	100	0	100	0
sheep owning	43.3	56.7	30.0	70.0
goat owning	33.3	66.7	23.3	76.7
donkey owning	31.0	69.0	26.7	73.3
horse owning	36.6	63.4	20.0	80.0
Detect diarrhea in their livestock	36.7	63.3	46.7	53.3

5. Discussion

Sampled springs considered in this study were located in the uplands nearby the settlement area. Spring 1 and spring 2 are close by while spring 3 is further away from both springs (Fig.1). All springs were utilized more or less similarly. People utilized water from the springs by drawing with cans that might be unclean. Livestock drink directly at the same water points, not provided with water troughs. Average indicator bacteria counts of six sampling rounds at all spring sites were not uniform. In most cases the highest average counts of indicators were detected in spring 3, but in all springs the water was beyond the acceptable level for drinking in all six sampling rounds (Table 2 and 3).

Residents at the sampling points of the river drew water using cans and cups for drinking and domestic consumption without any treatment (Table 9). In addition, they washed their clothes and body only a little bit away from the collection points which could be sources of contamination. Livestock have free access and directly get into the river to drink; hence there is an opportunity of animal defecating and urinating inside water points. Moreover, as the road used for transport cross the river without any bridge people, livestock and vehicles cross the river by entering it, which is another potential source of contamination of Legebatu River. The lowest average count of indicators in the river was detected at the upper river site, possibly due to less intensive use and hence reduced pollution and impacts incurred by men and livestock. Highest counts were detected at river site 3, followed by river site 1, and river site 2 (Table 2 and 3).

Contamination levels of all indicators at each site over the six sampling rounds were not consistent over time. In most cases the highest average indicator bacteria concentrations in both river and springs were observed in January and February. This might be due to elevated surface flow into the water sources as these months are considered as short rainy season. In contrast, lower average counts of indicator bacteria were mostly recorded in December, a month of the dry season in which flood contamination would not be expected.

Average counts of total coliform bacteria per 100ml were 940.0±138.6 in spring 1, 950.8±194.9 in spring 2, 1447.0±163.3 in spring 3 and 884.2±210 in river site 1, 812.2±163.7 in river site 2, 831.8±189.3 in river site 3 and 198.3±46.9 in the upper river site (Table 2). All samples from all water sources in this study were contaminated with total coliforms, except in the upper river site, where 91.7% of the samples were contaminated (Table 1). Out of these, 100% of the samples from spring 1, spring 2, spring 3, river site 1, river site 3, 83.3% of the samples from river site 2 and 91.7% of the upper river site samples (Table 3) had unacceptable levels of total coliforms according to the suggested criteria for drinking (WHO, 2004a; FDRE, MoH, 2002).

In a study conducted on unprotected springs in North-Gondar, Ethiopia, Mengesha and his co-workers demonstrated that fifty percent of the samples had a coliform count of 180 and above /100 ml and the lowest coliform count was 13 coliform /100 ml (Mengesha *et al*., 2004), which are lower than the present study of Yubdo-Legebatu that was 198.3 coliform /100 ml. In another study in South Wello, Ethiopia, Atsnaf demonstrated that two thirds of the samples from protected springs were contaminated with total coliforms (Atsnaf, 2006). This was less than in the present study, where all water sources were contaminated with total coliform. This difference can possibly be attributed to the protection around the springs as usually is the case in Wello province and not in Yubdo-Legebatu. In contrast, results of monitoring six sampling stations in the Geum River in Korea showed average concentrations of total coliforms ranging from 1670 to 8510 cfu/100 ml (Geonha *et al*., 2005). That is higher than the present study and the possible reasons for this variation might be differences in dilution and sources of contaminants.

ANOVA of total coliform concentration among all sites showed that there was a significant difference ($p< 0.01$) in the average counts of TC between the sampling sites (Table 4). Total coliforms in spring 3 were significantly higher than in all other sites. Sanitary inspection (Table 9) and personal observation revealed that a high number of people use this site. Moreover, there is a big tree over the site and debris falls into the water, sometimes covering it (Figure 2: spring 3). In addition drawing water is done using unclean cups and cans, while there is also open access for livestock and wildlife. All these factors might be possible reasons for the high concentrations in total coliforms in this site.

Average counts of fecal coliforms per 100 ml were 405.8±42.6 in spring 1, 365.0±83.9 in spring 2, 741.7±129.8 in spring 3 and 502.5±119.1 in river site 1, 419.2±133.4 in river site 2, 495.8 ± 151.2 in river site 3 and 75.8 ± 24.1 in the upper river site (Table 2). All samples from all water sources were contaminated with fecal coliforms, except for river site 2 and the upper river site, of which 91.7% and 83.3% of samples were, contaminated respectively (Table 1). Moreover, 100% of samples from spring 1, spring 2, spring 3 and river site 1, river site 2, 91.7% of river site 3 and 83.3% of samples from the upper river site (Table 3) had unacceptable level of fecal coliforms according to the suggested criteria for drinking water (WHO, 2004a; FDRE, MoH, 2002). Contamination levels of 75% by coliforms, especially *E.coli*, among the samples taken from unprotected springs in North-Gondar, Ethiopia, have been reported by Mengesha and his colleagues (Mengesha *et al*., 2004). On the other hand, a study made to monitor six sampling stations in the Geum river had shown average concentrations ranging from 170 to 4450 cfu/100 ml (Geonha *et al*., 2005), which was a very high value, unlike this study.

ANOVA of fecal coliform concentrations among all sites showed that there was a highly significant difference ($p < 0.01$) in the average counts of TTC among all water sites (Table 5). Mean fecal coliform levels in spring 3 were significantly higher than in other sites. Fecal coliforms are indicators of fecal contamination. Hence, categorizing the site in terms of risk to human health, the majority (81.2%) of sampled water sources in the study area were at high risk, 16.6% at intermediate risk and only 2.4% at reasonable quality. However, none of the water sources were safe to human consumption. This significant proportion of contamination was also supported by the result of the sanitary risk assessment based on questioning 30 households. In all cases interviewed people responded that they did not use latrines but instead they indicated the common use of open areas nearby water sources. Similarly, all households that graze their livestock nearby water sources indicated a failure of fencing watering points to prevent the entrance of animals (Table 9).

Average counts of fecal streptococci per 100 ml were 120.8±23.1 in spring 1, 100.8±48.1 in spring 2, 411.7±139.0 in spring 3 and 90.0±38.7 in river site 1, 168.3±61.3 in river site 2,

94.2±48.8 in river site 3 and 30.8±13.9 in the upper river site (Table 2). Hundred percent of the samples from spring 1, spring 2, and river site 2; 91.7% of samples from spring 3; 75 % samples from river site 1 and 58.3% of those from the upper river site were contaminated with fecal streptococci (Table 1). Out of these, 91.7% of samples from spring 1 and spring 3, 66.8% of those from river site 1 and river site 3, 83.3% of samples from spring 2 and river site 2 and 58.3% of samples from the upper river site (Table 3) had unacceptable levels of fecal streptococcus according to the criteria for drinking water (WHO, 2004a; FDRE, MoH, 2002).

ANOVA of fecal *Streptococcus* concentrations among all sites showed that there was a highly significant difference ($p < 0.01$) in the average counts of FS among all water sites (Table 6). The mean concentration of FS in Spring 3 was significantly higher than in all other sites.

Based on the concept of using ratios between fecal coliform and fecal *Streptococcus* counts to determine the main sources of pollution (Coyne and Howell, 1994), ratios of FC/FS were computed for the study area as summarized in Table 2. only for those cases where streptococci were equal and above 100cfu/100ml (APHA, 1998).

Fecal coliform - fecal streptococci ratios in water sources that had streptococci counts equal and above 100cfu/100ml showed that in 100% of indicated enteric contamination originated from domestic animal wastes. The origin of the bacteria was observed to be livestock wastes, from the numerous settlements situated throughout the watershed characterized by existence of the livestock that have free access to the water sources, graze nearby water points and improper sanitary facility. A similar study in Lebanon and Syria to quantify the fecal coliform to fecal streptococcus ratios sampled for three periods were 1.4 in spring and 1.1, 6.7 and 16.7 in river (Monzer *et al*., 2005), the interpretation of which concurs with this study.

Pearson correlations for microbiological counts and water temperature, turbidity and pH suggested that microbiological counts had strong and positive relationship with temperature ($r = 0.28$, $p < 0.01$) and turbidity ($r = 0.21$, $p = 0.0008$) and a significant but negative relationship with pH ($r = -0.15$, $p = 0.012$). Average temperature of springs and river sites in this study was

17.82±0.13°C and 17.77±0.14°C, turbidity 12.61±0.99 NTU and 6.33 ±0.76 NTU and pH 7.56±0.16 and 8.08±0.11, respectively (Table 2). The highest records were observed in spring 3: total coliform concentration was 1447/100 ml, fecal coliform 741.7/100 ml and fecal *Streptococcus* 411.7/100 ml at temperature 18.00±0.1, turbidity 13.83±0.8 and pH 7.26±0.2. The lowest counts were recorded in the undisturbed river site: 198.3 TC/100 ml, 75.8 FC/100 ml and 30.8 FS/100 ml at temperature 16.82±0.2, turbidity 7.17±0.9 and pH 8.0±0.1. On average, the occurrence of coliform bacteria was significantly higher when water temperatures were above 15°C in line with findings by LeChevallier (2003). The pH values were identified slightly alkaline, the pH value advised by the World Health Organization (2004a) and Ethiopian Ministry of Water Resources (FDRE, MoWR, 2002) is 6.5 - 8.5, and so all samples were in the acceptable range. However, bacterial cell die-off be expected at extreme pH <4.5 or >8.2 (James, 1999), hence the pH values in the present study favor the growth of microorganisms.

According to the Food Act (1983), the maximum level of turbidity permitted for drinking water is 5 NTU, while WHO (1998) stated that drinking water is best consumed with NTU less than 1 for health purposes. The results for turbidity analyses suggest that the appearance of water with a turbidity of more than 5 NTU is usually not acceptable to consumers. The consumption of highly turbid water may constitute a health risk as excessive turbidity can protect pathogenic microorganisms from the effect of disinfectants, and also stimulate the growth of bacteria (Zvikomborero, 2005). This is confirmed in this study where results of the measured physico-chemical quality of water in Yubdo-Legebatu shows high turbidity together with high bacterial contamination in both springs and river sites.

Total coliforms, fecal coliforms and fecal Streptococci indicator bacteria were isolated from all livestock feces samples and were found to be at high concentrations in most cases (Table 8). The highest median and maximum concentrations of total coliforms in the livestock feces were 3.25 $x10^7$ cfu/g and 4.3 $x10^7$ cfu/g, from goat and sheep fecal samples at river site 2 and spring 2, respectively. The highest median and maximum counts of fecal coliforms were 2.05 $x10^7$ cfu/g

and 2.4 x10^7 cfu/g, from goat and sheep fecal samples at river site 2 and spring 2, respectively. The highest median and maximum concentrations of fecal *Streptococcus* were 1.6 x10^7 cfu/g and 3 x10^7 cfu/g, from cattle fecal samples at river site 1. Hence, results of the indicator concentrations in livestock feces and FC/FS ratios (Table 2) support that the possible main sources of contamination of Yubdo-Legebatu PA water sources could be livestock wastes. Cattle that constitute the largest herd size could be share the highest load of contaminants to the water sources among the other livestock species in the PA.

In this study among the livestock considered the values of fecal coliforms measured per gram wet weight of fecal matter were 1 ×10^6-1.6 ×10^7 for cattle, 4 ×10^6- 4.3 ×10^7 for sheep and 3 ×10^6- 2.2 ×10^7 for horses. These results partly concur with that of Cox and his co-workers (2005), whose study in Australia on fecal coliforms/g wet weight feces found values ranging from 1.3 x10^3 to 8.5 x10^6 for adult cattle, 1.0 x10^5 to 1.9 x10^8 for sheep and 4.0 x10^3 to 3.3 x10^6 for horses. Mara (1974) also reported fecal coliform concentrations of 1.6x10^7 and 2.3x10^5 per gram of sheep and cattle feces, comparable to what has been reported in this study. However, among the livestock considered in this report, cattle had the highest median (1.6 x10^7 cfu/g) and maximum concentration (3 x10^7 cfu/g) of fecal *Streptococcus*, recorded at river site 1, while, sheep feces were found to have 1.1 x10^7 fecal streptococci per gram.

Slightly higher values for concentrations of indicator bacteria were found in livestock feces samples in this study as compared to the two studies illustrated above. This might be attributed to the variations in livestock management. Sinton and his co-workers (1998) reported the entry of even a small portion of livestock feces into the rural water ways would provide a high non-point source microbial load, and a potentially important reservoir for zoonotic pathogens. Hence, the livestock considered in the present study probably contributes significantly to the pollution of the water sources in Yubdo-Legebatu.

6. Conclusions and Recommendations

6.1. Conclusions

Based on the research findings, the following conclusions have been drawn:

- Bacteriological quality of the sampled water sources in Yubdo-Legebatu did not meet national or international guidelines for drinking water.
- The overall bacterial count and sanitary risk factor assessment indicated that the majority of water sources in Yubdo-Legebatu could be classified as high risk, while some were at intermediate risk and very few water points had reasonable quality.

- High counts of indicator organisms in all sampled water sources of the study area suggested the presence of pathogenic organisms that constitute a threat to anyone consuming these water sources.
- The contamination of these water sources with enteric organisms can be explained in part by absence of fencing of watering points that could prevent the entrance of animals, livestock grazing nearby water sources, people's open area defecation, drawing of water with unclean cups and agricultural activities nearby water sources.
- Fecal coliform - fecal streptococci ratios in this study showed that while human contribution was in place the main sources of contaminants of the water sources could be livestock wastes.
- Concentrations of bacterial indicators in the livestock feces around the six sampling sites were higher than what has been reported in the literature.
- Finally, the baseline information generated from this study may contribute to develop similar programs for further studies.

6.2. Recommendations

Based on the results and conclusions of this study, the following recommendations are formulated:

- As indicator bacterial counts in all sampled water sites have exceeded the guidelines set for human use there is, clearly, an urgent need to develop safe water supplies and basic sanitation in the area.
- Wastes from both livestock and human were found to be causes of the problem, so minimizing fecal contamination of water with livestock and human wastes will have a dramatic impact on reducing water sources pollution in the study area.

- Priority should be given to create awareness in the community of measures to improve hygiene, such as to develop a habit of using latrines, which is indispensable for improved water quality. Defecation of people around water points should be corrected.
- Measures have to be taken to divide the water sources for human and livestock uses.
- Entrance of animals into water sources for human use should be protected by fencing the surroundings.
- Springs should be cleaned by emptying them and removing any sediment and vegetation. Constructing covers over springs will protect them from free inflow of contaminants.
- The sand filtration pot tested in the same site showed good results. It is a promising method for improving water quality at household level (Ephrem, 2007). Enabling the community to develop and use this method or other home water treatment techniques is crucial.
- Protection of water sources accompanied by sanitation and hygiene promotion programs can improve the hygiene quality of rural water sources, where disinfection is not feasible. Hygiene education is an essential part of water supply and sanitation projects.
- Future studies are needed to determine the seasonal variations in the contamination level of the water sources, to quantify pathogen loads in both the water sources and livestock feces and to develop risk-reducing livestock management systems.

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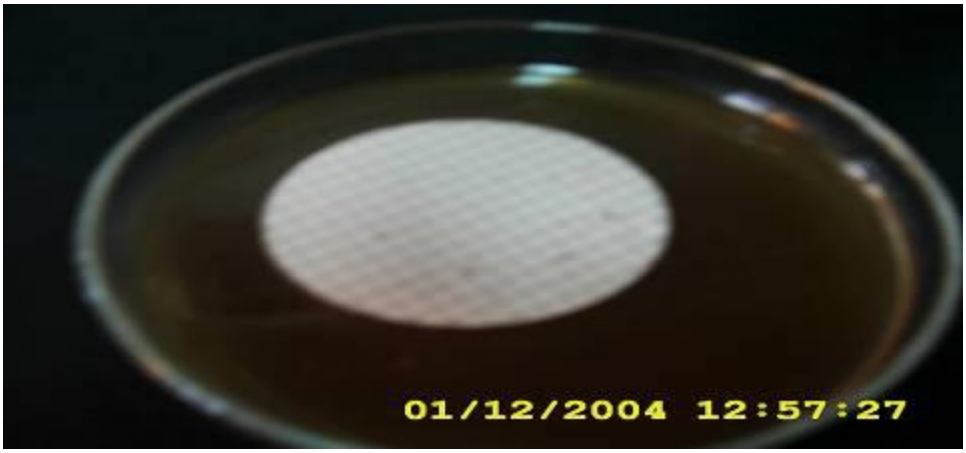
Annex 1. Figure of TC, TTC and FS

TC

TTC



FS

Annex -2. Table showing total coliform (tc, in CFU) Fecal coliform (fc, in CFU) and fecal *Streptococcus* (fs, in CFU), temperature (^{o}C) turbidity (NTU) and pH (pH meter) measurements in the six sampling rounds.

Obs	sites	date	rep	count	temp	turb	PH	bacteria
1	RU	d1	1	150	16.0	6	8.00	tc
2	RU	d1	2	160	16.0	6	8.00	tc
3	RS1	d1	1	530	17.5	6	8.10	tc
4	RS1	d1	2	220	17.5	6	8.10	tc
5	RS2	d1	1	620	17.8	4	8.39	tc
6	RS2	d1	2	270	17.8	4	8.39	tc
7	RS3	d1	1	240	18.0	3	7.89	tc
8	RS3	d1	2	490	18.0	3	7.89	tc
9	SS1	d1	1	890	16.5	4	7.62	tc

10	SS1	d1	2	880	16.5	4	7.62	tc	
11	SS2	d1	1	620	17.0	16	8.27	tc	
12	SS2	d1	2	500	17.0	16	8.27	tc	
13	SS3	d1	1	370	17.8	9	6.79	tc	
14	SS3	d1	2	810	17.8	9	6.79	tc	
15	RU	d2	1	60	16.5	3	7.72	tc	
16	RU	d2	2	30	16.5	3	7.72	tc	
17	RS1	d2	1	70	17.6	5	8.52	tc	
18	RS1	d2	2	430	17.6	5	8.52	tc	
19	RS2	d2	1	30	17.8	2	7.98	tc	
20	RS2	d2	2	80	17.8	2	7.98	tc	
21	RS3	d2	1	310	18.0	4	8.00	tc	
22	RS3	d2	2	440	18.0	4	8.00	tc	
23	SS1	d2	1	1860	17.5	6	7.92	tc	
24	SS1	d2	2	890	17.5	6	7.92	tc	
25	SS2	d2	1	1990	17.6	17	7.60	tc	
26	SS2	d2	2	480	17.6	17	7.60	tc	
27	SS3	d2	1	1790	17.8	16	8.25	tc	
28	SS3	d2	2	.	17.8	16	8.25	tc	.
29	RU	d3	1	0	16.2	4	7.88	tc	
30	RU	d3	2	70	16.2	4	7.88	tc	
31	RS1	d3	1	900	18.0	8	7.87	tc	
32	RS1	d3	2	1200	18.0	8	7.87	tc	
33	RS2	d3	1	750	18.0	6	8.10	tc	
34	RS2	d3	2	1270	18.0	6	8.10	tc	
35	RS3	d3	1	1230	18.5	7	8.20	tc	
36	RS3	d3	2	1980	18.5	7	8.20	tc	
37	SS1	d3	1	860	17.7	4	8.10	tc	
38	SS1	d3	2	630	17.7	4	8.10	tc	
39	SS2	d3	1	1190	17.8	17	8.46	tc	
40	SS2	d3	2	400	17.8	17	8.46	tc	
41	SS3	d3	1	1610	17.6	17	7.21	tc	
42	SS3	d3	2	1480	17.6	17	7.21	tc	
43	RU	d4	1	170	16.9	8	8.20	tc	
44	RU	d4	2	150	16.9	8	8.20	tc	
45	RS1	d4	1	230	17.3	6	8.30	tc	
46	RS1	d4	2	190	17.3	6	8.30	tc	
47	RS2	d4	1	1860	17.8	8	7.86	tc	
48	RS2	d4	2	1620	17.8	8	7.86	tc	
49	RS3	d4	1	760	18.1	5	8.27	tc	
50	RS3	d4	2	410	18.1	5	8.27	tc	
51	SS1	d4	1	990	18.0	5	8.00	tc	
52	SS1	d4	2	1920	18.0	5	8.00	tc	
53	SS2	d4	1	920	18.0	17	8.40	tc	

54	SS2	d4	2	1090	18.0	17	8.40	tc	
55	SS3	d4	1	2030	17.9	15	7.81	tc	
56	SS3	d4	2	.	17.9	15	7.81	tc	
57	RU	d5	1	440	17.5	10	8.21	tc	
58	RU	d5	2	400	17.5	10	8.21	tc	
59	RS1	d5	1	2030	18.5	7	8.49	tc	
60	RS1	d5	2	1000	18.5	7	8.49	tc	
61	RS2	d5	1	980	18.6	4	8.35	tc	
62	RS2	d5	2	806	18.6	4	8.35	tc	
63	RS3	d5	1	.	18.7	4	8.29	tc	.
64	RS3	d5	2	1970	18.7	4	8.29	tc	
65	SS1	d5	1	390	18.0	5	6.98	tc	
66	SS1	d5	2	700	18.0	5	6.98	tc	
67	SS2	d5	1	100	18.0	15	7.31	tc	
68	SS2	d5	2	290	18.0	15	7.31	tc	
69	SS3	d5	1	1400	18.3	14	6.70	tc	
70	SS3	d5	2	1330	18.3	14	6.70	tc	
71	RU	d6	1	280	17.8	12	8.00	tc	
72	RU	d6	2	470	17.8	12	8.00	tc	
73	RS1	d6	1	1830	18.2	11	7.34	tc	
74	RS1	d6	2	1980	18.2	11	7.34	tc	
75	RS2	d6	1	570	18.0	8	8.10	tc	
76	RS2	d6	2	890	18.0	8	8.10	tc	
77	RS3	d6	1	530	19.2	11	7.81	tc	
78	RS3	d6	2	790	19.2	11	7.81	tc	
79	SS1	d6	1	820	18.2	22	7.10	tc	
80	SS1	d6	2	450	18.2	22	7.10	tc	
81	SS2	d6	1	1890	18.4	16	6.71	tc	
82	SS2	d6	2	1940	18.4	16	6.71	tc	
83	SS3	d6	1	1670	18.6	12	6.82	tc	
84	SS3	d6	2	1980	18.6	12	6.82	tc	
85	RU	d1	1	70	16.0	6	8.00	fc	
86	RU	d1	2	100	16.0	6	8.00	fc	
87	RS1	d1	1	250	17.5	6	8.10	fc	
88	RS1	d1	2	100	17.5	6	8.10	fc	
89	RS2	d1	1	110	17.8	4	8.39	fc	
90	RS2	d1	2	180	17.8	4	8.39	fc	
91	RS3	d1	1	130	18.0	3	7.89	fc	
92	RS3	d1	2	230	18.0	3	7.89	fc	
93	SS1	d1	1	470	16.5	4	7.62	fc	
94	SS1	d1	2	560	16.5	4	7.62	fc	
95	SS2	d1	1	310	17.0	16	8.27	fc	
96	SS2	d1	2	220	17.0	16	8.27	fc	
97	SS3	d1	1	130	17.8	9	6.79	fc	

98	SS3	d1	2	530	17.8	9	6.79	fc
99	RU	d2	1	0	16.5	3	7.72	fc
100	RU	d2	2	30	16.5	3	7.72	fc
101	RS1	d2	1	30	17.6	5	8.52	fc
102	RS1	d2	2	200	17.6	5	8.52	fc
103	RS2	d2	1	0	17.8	2	7.98	fc
104	RS2	d2	2	10	17.8	2	7.98	fc
105	RS3	d2	1	230	18.0	4	8.00	fc
106	RS3	d2	2	10	18.0	4	8.00	fc
107	SS1	d2	1	630	17.5	6	7.92	fc
108	SS1	d2	2	430	17.5	6	7.92	fc
109	SS2	d2	1	700	17.6	17	7.60	fc
110	SS2	d2	2	280	17.6	17	7.60	fc
111	SS3	d2	1	980	17.8	16	8.25	fc
112	SS3	d2	2	1760	17.8	16	8.25	fc
113	RU	d3	1	0	16.2	4	7.88	fc
114	RU	d3	2	30	16.2	4	7.88	fc
115	RS1	d3	1	570	18.0	8	7.87	fc
116	RS1	d3	2	710	18.0	8	7.87	fc
117	RS2	d3	1	330	18.0	6	8.10	fc
118	RS2	d3	2	600	18.0	6	8.10	fc
119	RS3	d3	1	500	18.5	7	8.20	fc
120	RS3	d3	2	400	18.5	7	8.20	fc
121	SS1	d3	1	410	17.7	4	8.10	fc
122	SS1	d3	2	270	17.7	4	8.10	fc
123	SS2	d3	1	100	17.8	17	8.46	fc
124	SS2	d3	2	200	17.8	17	8.46	fc
125	SS3	d3	1	1060	17.6	17	7.21	fc
126	SS3	d3	2	910	17.6	17	7.21	fc
127	RU	d4	1	70	16.9	8	8.20	fc
128	RU	d4	2	40	16.9	8	8.20	fc
129	RS1	d4	1	170	17.3	6	8.30	fc
130	RS1	d4	2	130	17.3	6	8.30	fc
131	RS2	d4	1	1650	17.8	8	7.86	fc
132	RS2	d4	2	870	17.8	8	7.86	fc
133	RS3	d4	1	460	18.1	5	8.27	fc
134	RS3	d4	2	1940	18.1	5	8.27	fc
135	SS1	d4	1	380	18.0	5	8.00	fc
136	SS1	d4	2	570	18.0	5	8.00	fc
137	SS2	d4	1	200	18.0	17	8.40	fc
138	SS2	d4	2	390	18.0	17	8.40	fc
139	SS3	d4	1	590	17.9	15	7.81	fc
140	SS3	d4	2	850	17.9	15	7.81	fc
141	RU	d5	1	130	17.5	10	8.21	fc

142	RU	d5	2	310	17.5	10	8.21	fc
143	RS1	d5	1	810	18.5	7	8.49	fc
144	RS1	d5	2	1300	18.5	7	8.49	fc
145	RS2	d5	1	500	18.6	4	8.35	fc
146	RS2	d5	2	350	18.6	4	8.35	fc
147	RS3	d5	1	550	18.7	4	8.29	fc
148	RS3	d5	2	1020	18.7	4	8.29	fc
149	SS1	d5	1	410	18.0	5	6.98	fc
150	SS1	d5	2	400	18.0	5	6.98	fc
151	SS2	d5	1	100	18.0	15	7.31	fc
152	SS2	d5	2	120	18.0	15	7.31	fc
153	SS3	d5	1	300	18.3	14	6.70	fc
154	SS3	d5	2	170	18.3	14	6.70	fc
155	RU	d6	1	40	17.8	12	8.00	fc
156	RU	d6	2	90	17.8	12	8.00	fc
157	RS1	d6	1	780	18.2	11	7.34	fc
158	RS1	d6	2	980	18.2	11	7.34	fc
159	RS2	d6	1	230	18.0	8	8.10	fc
160	RS2	d6	2	200	18.0	8	8.10	fc
161	RS3	d6	1	260	19.2	11	7.81	fc
162	RS3	d6	2	220	19.2	11	7.81	fc
163	SS1	d6	1	210	18.2	22	7.10	fc
164	SS1	d6	2	130	18.2	22	7.10	fc
165	SS2	d6	1	870	18.4	16	6.71	fc
166	SS2	d6	2	890	18.4	16	6.71	fc
167	SS3	d6	1	720	18.6	12	6.82	fc
168	SS3	d6	2	900	18.6	12	6.82	fc
169	RU	d1	1	0	16.0	6	8.00	fs
170	RU	d1	2	20	16.0	6	8.00	fs
171	RS1	d1	1	0	17.5	6	8.10	fs
172	RS1	d1	2	0	17.5	6	8.10	fs
173	RS2	d1	1	50	17.8	4	8.39	fs
174	RS2	d1	2	30	17.8	4	8.39	fs
175	RS3	d1	1	10	18.0	3	7.89	fs
176	RS3	d1	2	20	18.0	3	7.89	fs
177	SS1	d1	1	20	16.5	4	7.62	fs
178	SS1	d1	2	30	16.5	4	7.62	fs
179	SS2	d1	1	10	17.0	16	8.27	fs
180	SS2	d1	2	10	17.0	16	8.27	fs
181	SS3	d1	1	70	17.8	9	6.79	fs
182	SS3	d1	2	430	17.8	9	6.79	fs
183	RU	d2	1	0	16.5	3	7.72	fs
184	RU	d2	2	0	16.5	3	7.72	fs
185	RS1	d2	1	0	17.6	5	8.52	fs

186	RS1	d2	2	20	17.6	5	8.52	fs
187	RS2	d2	1	10	17.8	2	7.98	fs
188	RS2	d2	2	10	17.8	2	7.98	fs
189	RS3	d2	1	0	18.0	4	8.00	fs
190	RS3	d2	2	10	18.0	4	8.00	fs
191	SS1	d2	1	120	17.5	6	7.92	fs
192	SS1	d2	2	210	17.5	6	7.92	fs
193	SS2	d2	1	40	17.6	17	7.60	fs
194	SS2	d2	2	60	17.6	17	7.60	fs
195	SS3	d2	1	1570	17.8	16	8.25	fs
196	SS3	d2	2	390	17.8	16	8.25	fs
197	RU	d3	1	0	16.2	4	7.88	fs
198	RU	d3	2	20	16.2	4	7.88	fs
199	RS1	d3	1	420	18.0	8	7.87	fs
200	RS1	d3	2	300	18.0	8	7.87	fs
201	RS2	d3	1	400	18.0	6	8.10	fs
202	RS2	d3	2	100	18.0	6	8.10	fs
203	RS3	d3	1	600	18.5	7	8.20	fs
204	RS3	d3	2	100	18.5	7	8.20	fs
205	SS1	d3	1	180	17.7	4	8.10	fs
206	SS1	d3	2	160	17.7	4	8.10	fs
207	SS2	d3	1	100	17.8	17	8.46	fs
208	SS2	d3	2	160	17.8	17	8.46	fs
209	SS3	d3	1	860	17.6	17	7.21	fs
210	SS3	d3	2	800	17.6	17	7.21	fs
211	RU	d4	1	20	16.9	8	8.20	fs
212	RU	d4	2	60	16.9	8	8.20	fs
213	RS1	d4	1	60	17.3	6	8.30	fs
214	RS1	d4	2	30	17.3	6	8.30	fs
215	RS2	d4	1	570	17.8	8	7.86	fs
216	RS2	d4	2	550	17.8	8	7.86	fs
217	RS3	d4	1	60	18.1	5	8.27	fs
218	RS3	d4	2	20	18.1	5	8.27	fs
219	SS1	d4	1	110	18.0	5	8.00	fs
220	SS1	d4	2	90	18.0	5	8.00	fs
221	SS2	d4	1	20	18.0	17	8.40	fs
222	SS2	d4	2	20	18.0	17	8.40	fs
223	SS3	d4	1	620	17.9	15	7.81	fs
224	SS3	d4	2	100	17.9	15	7.81	fs
225	RU	d5	1	0	17.5	10	8.21	fs
226	RU	d5	2	10	17.5	10	8.21	fs
227	RS1	d5	1	30	18.5	7	8.49	fs
228	RS1	d5	2	10	18.5	7	8.49	fs
229	RS2	d5	1	170	18.6	4	8.35	fs

230	RS2	d5	2	50	18.6	4	8.35	fs
231	RS3	d5	1	10	18.7	4	8.29	fs
232	RS3	d5	2	30	18.7	4	8.29	fs
233	SS1	d5	1	110	18.0	5	6.98	fs
234	SS1	d5	2	10	18.0	5	6.98	fs
235	SS2	d5	1	100	18.0	15	7.31	fs
236	SS2	d5	2	40	18.0	15	7.31	fs
237	SS3	d5	1	40	18.3	14	6.70	fs
238	SS3	d5	2	0	18.3	14	6.70	fs
239	RU	d6	1	80	17.8	12	8.00	fs
240	RU	d6	2	160	17.8	12	8.00	fs
241	RS1	d6	1	110	18.2	11	7.34	fs
242	RS1	d6	2	100	18.2	11	7.34	fs
243	RS2	d6	1	60	18.0	8	8.10	fs
244	RS2	d6	2	20	18.0	8	8.10	fs
245	RS3	d6	1	200	19.2	11	7.81	fs
246	RS3	d6	2	70	19.2	11	7.81	fs
247	SS1	d6	1	130	18.2	22	7.10	fs
248	SS1	d6	2	280	18.2	22	7.10	fs
249	SS2	d6	1	40	18.4	16	6.71	fs
250	SS2	d6	2	610	18.4	16	6.71	fs
251	SS3	d6	1	30	18.6	12	6.82	fs
252	SS3	d6	2	30	18.6	12	6.82	fs

d is day

Declaration

I, the undersigned, declared that this is my original work, has not been presented for a degree in this or any other University, and that all sources of materials used for the thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as University Advisor.

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