# ASSESSMENT OF THE MICROBIOLOGICAL AND PHYSICO-CHEMICAL QUALITY OF DRINKING WATER USED AT INSENO TOWN, SOUTHERN ETHIOPIA.

**M.Sc. THESIS**

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# HARAMAYA UNIVERSITY

# Assessment of the Microbiological and Physico-Chemical Quality of Drinking Water used at Inseno Town, Southern Ethiopia

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**MASTER OF SCIENCE IN MICROBIOLOGY**

**By**

**Elfinesh Oshemo**

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## Haramaya University
# APPROVAL SHEET

As thesis research advisors, we here by certify that we have read and evaluated this thesis prepared, under our guidance, by Elfinesh Oshemo entitled: **"Assessment of the Microbiological and Physico-Chemical Quality of Drinking Water Sources Used At Inseno Town,Southern Ethiopia**

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Final approval and acceptance of the thesis is contingent upon the submission of its final copy to the Council of Graduate studies (CGS) through the candidate's department or school graduate committee (DGC or SGC).

# DEDICATION

I dedicate this thesis to my beloved mother, Rehmet Hirpo and my father Oshemo Herefo, for nursing me with affections and love and for her and his dedicated partnership in the success of my life.

## STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bonafide work and all the sources of materials used for this thesis have been duly acknowledged. This thesis has been presented in partial fulfillment of the requirement of MSc degree at Haramaya University and deposited at the University Library to be made available to borrow under rules of the library. I solemnly declare that this thesis is not submitted to any other institutions in anywhere for the award of any academic degree, diploma or certificate. Secondly, brief quotations from this thesis are allowed without special permission provided that accurate acknowledgement of source is made. Requests for permission of extended quotation from the thesis or reproduction of the whole manuscript or the part may be granted by the Head of Dean of Graduate Studies or by the Head of Biology Department when in his/her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

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## BIOGRAPHICAL SKETCH

The author was born in September, 1986 at Inseno, Meskan Woreda in Gurega Zone of Southern Nations, Nationalities, and Peoples' Region, from her mother Rehmet Hirpo and her father Oshemo Herefo. She attended her elementary education at Enseno Primary & Junior Secondary School and secondary education at Butajira Senior Secondary School. She then joined Adigerat University in 2006 and graduated with BEd degree in Biology in July 2008EC.

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## LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| APHA | American Public Health Association |
| DPD | Diethyl-p-Phenylene Diamine |
| EPA | Environmental Protection Agency |
| ES | Ethiopian Standards |
| HPCR | Heterogeneity Polymerase Chain Reaction |
| IOS | International Organization for Standardization |
| MoH | Ministry of Health |
| MPN | Most Probable Number |
| MWR | Ministry of Water Resource |
| NTU | Nephelometric Turbidity Units |
| SPSS | Statistical Package for the Social Sciences |
| TRFLP | Terminal Restriction Fragment Length Polymorphism |
| UNICEF | United Nations Children Emergency Fund |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organization |

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# Assessment of the Microbiological and Physico-Chemical Quality of Drinking Water Sources Used at Enseno Town, Southern Ethiopia

## ABSTRACT

*In Ethiopia, most of the communicable diseases arised from unsafe and inadequate water supply, poor hygienie and lack of regular treatment of water. Thus, this study was conducted to assess the microbial and physico-chemical quality of drinking water in Inseno town Southern Ethiopia. The study was conducted from February 2018 to March 2018. A total of 28 water samples were collected from infected reservoir, tap water and well water sources for microbiological and physico-chemical analysis following standard techniques. The results indicated that all water samples in the study area were contaminated with coliform bacteria and Giardia cyst in case of well water while all water samples of reservoir and tap water in the study area were free of contamination with coliform bacteria and Giardia lmbalia cyst. However, about 8.3 % of the tap water samples showed a presumptive bacterial load of 2MPN/100 ml which is higher than the presumptive limit recommended for drinking water. From water samples analyzed for parasitological quality only about 3.57% of water samples taken from total well water were positive for Giardia lamblia cyst whereas negative Entamoiba histolytica and Cryptosporidium parvum in drinking water samples. Majority of the water samples analyzed did not meet the acceptable limit of bacteriological quality of drinking water of WHO guidelines. Physico-chemical parameters of well water show significant difference in Ph, turbidity and fluoride at 0.05 level of significance. In case of tap water Fluorid show significant difference at 5% level of significance. The temperature records from wells to tap water samples showed a higher measurement (22.13 $^{O}$C-24.8$^{O}$C) compared to the standard of <15$^{O}$C. In case of reservoir water, correlation of chlorine and hardness Show highly*

*positively significant at 0.01 level. And also in case of tap water, correlation of hardness with turbidity Show highly positively significant at 0.01 level. In case of well water sample, correlation of Fluorid with turbidity Show negative significance at 0.05 level. Most of the physico-chemical parameters were within WHO guidelines recommended for drinking water with the exception temperature exceeded the WHO standard of less than 15$^{o}$C .Generally, the mean values of total hardness for all water samples within the recommended values of WHO. While majority of water samples did not obtain pH with in the recommended limit of WHO (6.5-8.5).*

**Key words:** Coliform bacteria, MPN, Protozoan parasites, Residual Free Chlorine,Total hardness.

# 1. INTRODUCTION

Water is the only substance that exists naturally on Earth in all three physical states of matter: gas, liquid, and solid. It is a very precious resource of this planet as it is an established source of life. Water quality is a critical factor affecting human health and welfare. It has been stated that our existence is intimately connected with the quality of drinking water available to us. Studies showed that access to safe drinking water is a fundamental human need and therefore, a basic human right (Abera Kuime and Mohamed Ali, 2005; de Kok *et al*., 2001; Gundry *et al*., 2006).

In this regard, as stated by organizations like Water Aid Ethiopia (2010), WHO (2011), MOH (2011), and MOWE (2013), water intended for domestic uses should be free from toxic substances and microorganisms that are of health hazards. So, clean, safe, and adequate freshwater is vital to the survival of all living organisms and the smooth functioning of ecosystems, communities, and economies.

In many areas most of the drinking water is ground water, an important natural resource both in terms of yield and water quality. It is an important source of drinking water and is utilized in food and industrial processing. Groundwater is generally a preferred source for water supply compared to other sources because of its constant and good natural quality as well as relatively low capital cost of water supply system development. It, however, requires appropriate physical, chemical and biological treatment depending on the nature of existing pollutants before being supplied for domestic uses (ZinabuTebeje, 2012).

Contaminated water serves as a mechanism to transmit communicable diseases such as diarrhea, cholera, dysentery, typhoid and guinea worm infection. Studies showed that diseases caused by contaminated water consumption and poor sanitation practices are the leading causes of death for children worldwide (WHO, 2004; WHO, 2005). Several reports confirmed that water related-diseases not only remain a leading cause of mortality and morbidity worldwide but that the spectrum of the diseases is expanding and the incidence of many water-related microbial diseases is increasing (WHO, 2003; WHO//UNCEF, 2009).

WHO estimated that in 2008 diarrheal diseases claimed the lives of 2.5 million people (WHO, 2011). For children under five years, this burden is greater than the burden of HIV/AIDS and Malaria (Liu *et al*., 2012). Unsafe drinking water, lack of good sanitation and unhygienic practices are associated with high mortality and morbidity from human excreta and garbage related diseases (WHO, 2003). Some pathogens may contaminate the water at the source, but also occur during transportation, distribution, or handling of the water in households or other working places (WHO, 2004).

Access to safe drinking water is a fundamental human need and, therefore, a basic right. Contaminated water jeopardizes both the physical and social health of all people and it is an affront to human dignity (Craun *et al*., 2002). A significant proportion of the world's population uses potable water for drinking, cooking, personal and home hygiene (WHO, 2004). However, there has been a growing concern among the general public with respect to the safety and aesthetic qualities of potable water supplies. In extreme cases, customers may avoid aesthetically unacceptable but otherwise safe drinking water in favor of more pleasant but potentially unsafe source (WHO, 2011).

Potable water released into the distribution system becomes altered during its passage through pipes, open reservoirs, standing pipes and storage tanks. Bacteria may enter the distribution system through failure to disinfect water, intermittent service, excessive network leakages, corrosion of pipe parts and inadequate sewage disposal (Lee and Schwab, 2005). If the raw water is used before treatment, it presents a sanitary risk and may be unsafe (Nath, 2006). Treatment of raw water will result in a decrease in microbial load, with many distribution systems later experiencing an increase in bacterial numbers with distance away from the point of treatment. The deterioration in water quality occurs either because of re-growth of microorganisms in bio-films, which are formed on interior surfaces of water pipes, or because of back siphonage of contaminated water. Biological activity in

biofilms is controlled by nutrient content of water, temperature and residual chlorine (Gatel *et al.,* 2000).

Drinking water contaminated by human or animal excreta is the main cause of water-related diseases. The first such diseases identified were typhoid and cholera, and both remain a serious problem in many regions of the world. In addition, most of the enteric and diarrheal diseases caused by bacteria, parasites and viruses, such as cholera, giardiasis, typhoid, and rotaviruses are common. Among these, the most common causes of severe diarrheal diseases include rotavirus, pathogenic *E. coli*, *Campylobacter jejuni*, and protozoan parasites (WHO, 2011 and UNICEF, 2008).

Research findings identified that every year, more people die from the consequences of unsafe water than from all forms of violence, including war. According to WHO (2011), worldwide, more than 80% of human diseases are caused by unsafe water supply and inadequate sanitation practices. In a situation where there is no clean water and proper sanitation, millions of people would suffer from devastating diseases and millions of children would die due to water-borne diseases.

Ethiopia is one of the developing countries where only 52% and 28% of its population have access to safe water and sanitation coverage, respectively (MOWR, 2007). For this reason, 60-80% of the population suffers from water-borne and water-related diseases (FDRE/MOH, 2007). This burdens the country with enormous financial and social costs to take care of such a huge number of people suffering from these debilitating infections. In the country, 20% of the population lives in urban centers. Eighty percent of the inhabitants of cities enjoy the provision of safe water through centralized treatment plants and distribution system (UNESCO, 2004).

However, different reports showed that water sources and distribution systems are contaminated with water quality indicators such as turbidity, organic matter, and fecal

microorganisms. These bacteria indirectly determine the risk of ingesting pathogens with polluted water (APHA, 1998). The reports also showed that water sources and distribution systems of towns and rural communities alike have serious water quality problems. Assessment of the bacteriological and physico-chemical qualities of urban source water and tap water distribution systems in Akaki-Kaliti sub-city of Addis Ababa (Mengestayehu, 2007), Ziway town ( Kassahun, 2008), Bahir Dar town (Getnet, 2008), and Nazareth town (Adama) (Temesgen, 2009) showed contamination of water by indicator bacteria such as total coliforms, faecal coliforms and/or faecal streptococci.

The problems of contamination of urban water distribution systems are said to be diverse. According to GTZ (1995), wastes from improper sanitation (sewage) and agricultural and other activities make their way to the water distribution networks. Furthermore, break in the distribution system, inverse pumping of soil contaminants through interruption of the water supply, age and improper maintenance of the distribution system, low level of chlorine (treatment efficiency) usually compromise the integrity of the distribution system and the quality of potable water (Muyina and Ngeakani, 1998; Phiri *et al.*, 2005; Zambrlan *et al.*, 2007).

All these findings give conclusive evidence that water quality problems are rampant both with small-scale and large-scale water delivery systems in the country. This would pose high health risks to users unless prompt intervention is undertaken. This, therefore, necessitates the evaluation and putting in place of sustainable monitoring system to determine the water quality status of municipal and rural water distribution systems.

Inseno town is located in Southern Nations and Nationalities Regional State (SNNRS), where there is no environmental protection practice. One of the most serious problems encountered in the town is the extremely hazardous sanitary conditions caused by raw sewerage coming out of residential houses, health and service institutions. Collected excreta and garbage are often

transported in unhygienic conditions and dumped on the periphery of the town without any treatment. The World Health Organization Microbiological Guidelines (2004) and Federal Democratic Republic of Ethiopia, Ministry of Water Resources (2002) for drinking water recommend zero total coliform and thermtolerant/fecal coliform/100 ml of water and zero concentration of *Giardia* and *Cryptosporidium* cysts.

Although reports are available on the physico-chemical quality of drinking water and hygiene and sanitary practices at the major cities and towns of Ethiopia, there has been no previous study conducted in the selected study area regarding the physico-chemical and microbial quality of drinking water at the level of the reservoir, tap and household water tankers. Thus, it is of paramount importance to evaluate the quality of drinking water in relation to household water container and the distribution system.

This study is intended to fill this gap by evaluating the water quality of Inseno town based on the counts of currently accepted bacterial indicators of drinking water quality, the presence of protozoan parasites like *Giardia lamblia*, Enta*amoeba histolytica* and *Cryptosporidium parvum*; and the determination of the physico-chemical characteristics such as temperature, pH, turbidity and residual free chlorine from reservoirs to household water tankers. The data thus generated could be eventually be used to examine whether or not the water fulfills the requirements recommended by WHO and ES. Access to safe drinking water and basic sanitation and wastewater treatment facilities in cities are instrumental in achieving success in meeting the millennium's development goal targets. Hence, this study was expected to give a baseline information on drinking water quality of Inseno town, Southern Nations and Nationalities Regional People (SNNRP), Ethiopia.

The general objective of this study was to assess the physico-chemical and microbiological quality of water samples collected from different wells, water reservoirs and taps of Enseno town.

Specific objective of this study were to:

- Determine the levels of specific bacteriological indicator organisms in samples of drinking water obtained from wells, reservoirs and taps in Inseno town.
- Detect the presence of *Giardia lamblia, Entamoeba histolytica and Cryptosporidium parvum* in drinking water.
- Determine physico-chemical characteristics of drinking water (Temperature, pH, turbidity, residual free chlorine, fluoride and total hardness).

# 2. LITERATURE REVIEW

## 2.1. Human Health and Water Quality

Drinking water quality is becoming an issue of global human health concern, principally due to water contamination with pathogens and potentially toxic chemicals (Hooda, 2007). It has a strong impact on people's health because water is a means of transmission for many pathogenic microorganisms that cause diarrheal diseases.

Water quality is a relative term that relates the composition of water with effects of natural processes and human activities. Deterioration of drinking water quality arises from introduction of chemical compounds into the water supply system through leaks and cross connection (Napacho and Manyele, 2010). Access to safe drinking water and sanitation is a global concern. However, developing countries, like Ethiopia, have suffered from a lack of access to safe drinking water from improved sources and to adequate sanitation services (WHO, 2006).

In Ethiopia over 60 % of the communicable diseases are due to poor environmental health conditions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices (MOH, 2011). About 80 % of the rural and 20 % of urban population have no access to safe water. Three-fourth of the health problems of children in the country are communicable diseases arising from the environment, specially water and sanitation. Forty-six percent of less than 5 years mortality is due to diarrhea in which water related diseases occupy a high proportion. The Ministry of Health, Ethiopia estimated 6000 children die each day from diarrhea and dehydration (MOH, 2011).

The development of industry and agriculture created a number of environmental problems including air and water pollution with their serious effects on human health (Wang *et al.*, 2010; Patrick, 2003). Rapid industrialization and urbanization have resulted in elevated emission of

toxic heavy metals entering the biosphere (Nweke, 2009; Gazso, 2001). A strong relationship between contaminated drinking water with trace elements and the incidence of chronic diseases such as renal failure, liver cirrhosis, hair loss, and chronic anemia has been documented. Renal failure is related to the contamination of drinking water with Cd and Pb; liver cirrhosis to the contamination with Cu and molybdenum; hair loss to the contamination with Cr and Ni; and chronic anemia to the contamination with Cd and Cu (Johri*et al.,* 2010).

## 2.2. Microbial Contaminations of Drinking Water and Associated Diseases

Microorganisms transmitted in water generally grow in the intestines and leave the body with faces (WHO, 2006). They include bacteria, viruses, protozoa, and other organism's .such pathogens are often found in water, frequently as a result of fecal matter from sewage discharges, leaking septic tanks, and runoff from animal (EPA, 2002). Diarrheal disease, which are major water-borne diseases are prevalent in many countries, mainly due to inadequate sewage treatment. Disease-causing organisms (pathogens) transmitted via drinking water are predominantly of faecal origin (and therefore known as enteric pathogens) (Ashbolt*et al*., 2001; Hunter *et al*., 2002). Since the pioneering epidemiology in the 1850's, whereby the English physician John Snow established that cholera was waterborne (Panet*het al*., 1998), it is amassed a sound understanding of the transmission of various pathogens that cause diarrhoea and other diseases via drinking water (Hunter *et al*., 2002). Furthermore, the efficacy of drinking water treatment (traditionally by filtration and chlorination) to remove the bacterial pathogens responsible for cholera (*Vibrio cholerae*) and typhoid fevers (*Salmonella typhi* and *S. paratyphi*), is well indexed by the common faecal indicator bacterium *Escherichia coli* (*E. coli*), which is excreted in the faces of all warm-blooded animals and some reptiles (Edberg *et al*., 2000; Enriquez *et al*., 2001). There are however, many enteric pathogens that behave differently to *E. coli*, particularly with respect to disinfection resistance and environmental persistence (Ashbolt *et al*., 2001). Of particular concern are the

chlorine-resistant parasitic protozoa, such as the environmentally shed oocysts of *Cryptosporidium parvum* and various enteric viruses (Hambidge, 2001; Li *et al*., 2002). It is therefore important to match the appropriate indicator for the group of pathogen(s) of interest, noting that there is no universal indicator, as often assumed with thermo-tolerant (faecal) coliforms or *E. coli*.

## 2.3. Contamination of Drinking Water and its Sources

Access to safe water is a fundamental human need and, therefore, a basic human right. However, safe water is one of the most important felt needs in public health in developing countries (Sobsey and Bartram, 2003). An estimated 1.1 billion people worldwide do not have access to an improved water supply, and many more use unsafe, contaminated water from improved sources (CDC, 2008). Consequently more than 250,000 children under the age of five die each year from diarrhea mainly caused by water-borne pathogens (WHO, 2008). As a result, water-borne diseases are among the most wide spread cases of mortality and morbidity in Ethiopia.

In Ethiopia, only 42% of the population has access to an improved water supply, and only11% of the population has access to adequate sanitation services. In rural areas, these numbers drop even further below these figures (Anonymous, 2009). Most of the water sources in Ethiopia are heavily contaminated with environmental wastes such as human and animal excreta, washed by runoff from heavy precipitation (Anonymous, 2009). Most people collect water from shallow unprotected ponds which are also shared by animals. Others collect water from shallow wells. Both of these sources are subject to contamination as rain water washes waste from surrounding areas into the source.

Effluent form septic tanks that pollute natural soils and water by contaminating organisms are a serious concern (WHO, 2005). The number of families not serviced by sanitary sewage systems continues to increase with the development of suburban and rural areas. Septic tanks and subsurface absorption fields are usually employed for the disposal of domestic wastes in the absence of centralized facilities (WHO, 2005). In Ethiopia 85% of the population live in the rural area and have no access to sewerage systems. Moreover, 35% of the people that are living in the city and towns have no access to sewerage systems. From this fact, it is obvious that surface waters are contaminated with human wastes, i.e., urine and faeces (EEPA, 2006).

The rest of the people that live in the town use septic tanker or release the sewage into the streams through tube. These burdens lead to the pollution of surface and ground water bodies. When the water is used for household consumption, it creates severe public health problems. Typhoid, paratyphoid, infectious hepatitis and infant diarrhea are some of the epidemic diseases that occur due to this contamination. This sewage also creates toxic effect or promotes eutrophication on the water bodies and upset aquatic biota and ecosystems (EEPA, 2006).

## 2.4. Physico-Chemical Parameters

The physico-chemical water quality parameters are the ones that are contributed by climatological, hydrological and geological factors. They affect the bacteriological, chemical and physical components of water. Once a pathogen leaves the host environment, it exposes to a great diversity of habitats with very different physico-chemical and nutritional condition during the treatment, storage and distribution of drinking water. For instance proper chlorine dosage depends upon a number of factors including chlorine demand, residual, contact period, turbidity, and pH (Herrmann *et al*., 2003; WHO, 2004c). Turbidity, pH, temperature, and chlorine residual are widely accepted as other critical water quality parameters describing microbiological quality of drinking water. These are recommended in water monitoring

programs as they may influence disinfection efficiencies and microbial survival (FDRE/MOH, 2006). So long as the contact time is sufficient to ensure minimum contact time (CT) values, proper disinfection can also be determined by measuring the free chlorine residual, turbidity, and pH. However, microbiological monitoring should be performed to ensure disinfection efficiency (Hach, 2000).

### 2.4.1. pH

pH is most important in determining the corrosive nature of water. The lower pH value the higher is the corrosive nature of water. pH was positively correlated with electrical conductance and total alkalinity (Guptaa, 2009). The reduced rate of photosynthetic activity the assimilation of carbon dioxide and bicarbonates which are ultimately responsible for increase in pH, the low oxygen values coincided with high temperature during the summer month. Various factors bring about changes the pH of water. The higher pH values observed suggests that carbon dioxide, carbonate-bicarbonate equilibrium is affected more due to change in physico-chemical condition (Karanth, 1987).

### 2.4.2. Temperature

Cool water is generally more palatable than warm water and temperature will impact on the acceptability of a number of other inorganic constituents and chemical contaminants that may affect taste. High water temperature enhances the growth of microorganisms and may increase taste, odor, colour, and corrosion problems (WHO, 2006**)**. In analysis of the physico-chemical quality of drinking water, temperature is considered as a critical parameter. It has an impact on many reactions, including the rate of disinfectant decay and by-product formation (Volk *et al*., 2003). As the water temperature increases the disinfectant demand and byproduct formation, nitrification, microbial activity, algal growth, taste, and odor episodes, lead and copper solubility increases. Moreover, sand calcium carbonate precipitation increases (Venkateswara, 2011).

### 2.4.3. Turbidity

Turbidity is a measure of the relative clarity of water. In its 1984 Guidelines, WHO recommended turbidity should be maintained at less than 5NTU, but if water was disinfected it would be better to aim for values of less than 1 NTU. Drinking water with turbidity less than 1NTU was considered safe if it was disinfected by chlorine with free residual of 0.5mg/l at pH less than 8.0 (WHO, 1984). Turbidity in drinking water is caused by particulate matter that may be present from source water as a consequence of inadequate filtration (WHO, 2004a; 2004b).Turbid water can be microbiologically contaminated and indirectly constitute a health issue. Turbidity is closely related to total suspended solids (TSS), but also includes plankton and other organisms (WHO, 2006). Turbidity is also considered as indirect indicator for the presence of microbes (WHO, 2006) and, therefore, microbiological parameter is closely linked to the microbiological safety of drinking water (Murphy, 2007a). Therefore, turbidity has to be correlated with bacterial contamination, and the probable existence of pathogens that are of human health concern (Olson, 2004; Downie, 2005)

### 2.4.5. Fluoride

Fluoride is a chemical element that is found most frequently in groundwater and has become one of the most important toxicological environmental hazards globally. Fluoride is one of the elements necessary for human life. Deficiency or excess of fluoride in the environment is closely associated with human health outcomes (Zhang *et al*., 2003). Fluoride when consumed at less than 0.5 mg/L produces adverse health effects including dental caries, lack of formation of dental enamel, and deficiency of mineralization of bones, especially in children, whereas excessive intake of fluoride (>1 mg/L) may lead to loss of calcium from tooth matrix, aggravating cavity formation and dental fluorosis induction (WHO, 1996). Thus, fluoride possesses a relatively narrow range between beneficial and adverse effects. Consequently, the

risk of dental fluorosis increases more rapidly than the reduction in dental decay with rise of fluoride concentration. However, skeletal fluorosis occurs when the fluoride (>3 ppm) is present in drinking water and the water is being consumed for about 8–10 years (Nawlakhe and Bulusu, 1989).

### 2.4.6. Hardness

Hardness of water is an important consideration in determining the suitability of water for domestic and industrial uses. Hardness is caused by multivalent metallic cations and with certain anions present in the water to form scale. The principal hardness-causing cations are the divalent calcium, magnesium, strontium, ferrous ion and mangnous ions (Jitendra *et al.*, 2008).

### 2.4.7. Residual free chlorine

Chlorine has a number of advantages as a disinfectant, including its relative cheapness, efficacy and ease of measurement, both in the laboratory and in the field. An important additional advantage over some other disinfectants is that chlorine disinfectant residual assist in preventing recontamination during distribution, transport, and household storage of water. The absence of residual chlorine in the distribution system may, in certain circumstances indicate the possibility of post watched contamination. So chlorine is added to drinking water supplies for the purpose of destroying or deactivating disease-producing microorganisms. This is termed water disinfection. Chlorine is usually added to water in liquid form or as sodium or calcium hypochlorite. Maintaining an adequate level of residual chlorine is of great importance in terms of distribution water quality management (Houssein, 2003). The low chlorine dose allowing inadequately disinfected water into the distribution system (Hrudey, 2003). The effective concentration of chlorine required to disinfect water is chlorine demand plus the necessary germicidal concentration (Volk *et al*., 2002).

## 2.5. Microbiological Standards in Drinking Water

Although groundwater has historically been thought to be free of microbial contamination, studies have indicated that contaminated groundwater sources could result in waterborne diseases if consumed without prior treatment (Momba *et al*., 2006). Pathogenic bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Aeromonas hydrophili*, *Shigella dysenteria*, *Salmonella tyhimurium*, *Pseudomonas spp*. and *Klebsiella spp*. have been reported in groundwater sources (Momba and Notshe, 2003).

Many potential pathogens could be associated with water. Although methods are now available to enumerate the concentration of various specific bacterial contaminants in water, these methods are commonly complex and time consuming, and hence impractical for routine monitoring of water quality (Gadgil, 1998). Identifying pathogenic organisms in the water is extremely difficult, unreliable, and not routinely undertaken as a laboratory procedure (Martins *et al*., 1995). Some of the methods are extremely labor intensive, require long incubation periods, require special reagents, or are very expensive. Some pathogens and viruses have never been successfully propagated in the laboratory (EPA, 2006). A more expedient approach is to test for the presence of an indicator bacterial species that would signal fecal contamination. For example, presence of *E. coli,* common intestinal bacteria, indicates presence of fecal contamination and the possibility of contamination by pathogenic microorganisms. Because, the density of indicator organisms is much greater than the density of pathogenic organisms, indicator organisms are used to measure the degree of contamination (Barrell *et al.,* 2000).

Such an indicator organism must have the following characteristics: It (*a*) must be easily isolated and enumerated, (*b*) must be present in very large numbers in normal fecal matter of humans and other warm-blooded animals (potential carriers of human pathogens), (*c*) must be more resistant to disinfection than the pathogens, (*d* ) must not multiply in water, and its

persistence in water must be comparable to that of fecal pathogens, and (*e*) must be generally absent from other sources (e.g. vegetable matter, soils, etc) of bacteria coming in contact with water. Thus, the presence of the indicator organism will signal fecal contamination and possible presence of pathogens, and its absence (in pre-treated or post-treatment) water will suggest that the water is probably free of pathogens (Gadgil, 1998).

***Escherichia Coli***

*E. coli* is a member of faecal coliform group, being a more specific indicator for the presence of faecal contamination. In addition, *E. coli* conforms to taxonomic as well as functional identification criteria and is enzymatically distinguished by the lack of urease and presence of β-glucuronidase. One disadvantage associated with this organism as an indicator is that it has been consistently found in pristine tropical rain forest aquatic and plant systems as well as soils (Hazen *et al*., 1990; Lasalde*et al*., 2005). Additionally, it seems to survive for short periods in aquatic temperate environments (Borrego *et al.,* 1990; Arrabal*et al*., 1983). *E. coli* is the faecal indicator of choice used in WHO Guidelines for Drinking-water Quality (Geneva and Switzerland, 2008; Ainsworth and WHO, 2004) and several countries include this organism in their regulations as the primary indicator of faecal pollution (*i.e.*, Europe, USA). Although it has long been known that *E. coli* can cause disease in humans, the bacteria naturally occur in the lower part of the gut of warm-blooded animals (Bartram*et al*., 2000; Schierack *et al.*, 2007). Its role as an enteric pathogen has been reinforced with the discovery of *E. coli* O157:H7 associated with haemorrhagic enteritis and haemolytic uremic syndrome, that was responsible of producing several drinking water outbreaks, and some of them lack of β-glucuronidase activity (Hunter, 2003; During *et al*., 2005).

## 2.6. Protozoan Parasites (*Cryptosporidium parvum, Giardia intestinalis, Entamoeba histolytica)*

Several species of parasitic protozoa are transmitted through water, with *Giardia intestinalis* and *Entamoeba histolytica/dispar* being among the most important intestinal parasites worldwide. Morbidity, and particularly mortality, rates for *E. histolytica/dispar* are high, especially in non industrialized countries. A wide variety of free-living amoebae occur in water, but only *Naegleria fowleri* and *Acanthamoeba* spp. have been identified as pathogenic for man. *N. fowleri* may be present in thermally polluted waters and sporadically causes fatal primary amoebic meningoencephalitis; however, only one outbreak has been related to a drinking-water supply system (Marshall, 1997). Since *Cryptosporidium parvum*, *Giardia intestinalis* the parasites of primary concern in the area of drinking-water supply, and much information on waterborne transmission is available from recent research; it is on these three organisms that the remainder of this section concentrates.

***Cryptosporidium spp. and Giardia spp***.

*Cryptosporidium* is an obligate, intracellular, coccidian parasite with a complex life cycle including sexual and asexual replication. Thick-walled oocysts with a diameter of 4–6 μm are shed in faeces. The genus *Cryptosporidium* has about 13 species, with human infections predominantly caused by *C. hominis* and the cattle genotype of *C. parvum*. Other *Cryptosporidium* species have been reported to cause infrequent infections. *Cryptosporidium* was discovered to infect humans in 1976, and waterborne transmission was confirmed for the first time in 1984 (WHO, 2011).

*Giardia* is a flagellated protozoan that parasitizes the gastrointestinal tract of humans and certain animals. The genus *Giardia* consists of a number of species, but human infection (giardiasis) is usually assigned to *G. intestinalis*, also known as *G. lamblia* or *G. duodenalis.*

*Giardia* has a relatively simple life cycle consisting of a flagellate trophozoite that multiplies in the gastrointestinal tract and an infective thick-walled cyst that is shed intermittently but in large numbers in faeces. The trophozoites are bilaterally symmetrical and ellipsoidal in shape. The cysts are ovoid in shape and 8–12 μm in diameter (WHO, 2011).

*Cryptosporidium* and *Giardia* are ubiquitous in the aquatic environment and their transmission stages (oocysts and cysts, respectively) may remain viable for several months under a range of environmental conditions (Smith *et al*., 1995). In addition, *Cryptosporidium* oocysts and *Giardia* cysts are resistant to conventional disinfectants at the concentrations and exposure times commonly used, and their infectious doses in humans have been estimated to be as low as 30 oocysts for *Cryptosporidium* (DuPont *et al*., 1995) and 10 cysts for *Giardia* (Adam, 2001).

The prevalence and levels of both *Giardia* and *Cryptosporidium* in a water supply depends upon a variety of contributors and their associated activities performed in the catchment area. The potential contributors of *Giardia* cysts and *Cryptosporidium* oocysts in raw water are summarized in a review article by Smith *et al*. (1995). Contributors include municipal sewage, stormwater, domestic and wild animals.

Moreover, outbreaks caused by drinking water were attributed to several factors; contamination of the water source by heavy rainfall, snow melts or agricultural runoffs which wash parasites from land areas into surface water, and contamination of water wells and canals by soil or by dead animal‘s carcasses thrown into them (Mac Kenzie *et al.,* 1994). Moreover, uncovered water tanks are exposed to environmental factors, as excreta of birds and rodents that may harbor parasites (Karanis *et al*., 2007). It can also be attributed to inadequate or deficient treatment of drinking water (Richardson *et al*., 1991). The number and extent of outbreaks of waterborne diseases in developed countries show that their transmission by drinking water is a significant risk (Castro-Hermida *et al*., 2010). Knowledge of such

contributors to specific catchments can assist in the determination of sampling locations and analysis strategies.

Waterborne outbreaks of giardiasis have been associated with drinking-water supplies for over 30 years; at one stage, *Giardia* was the most commonly identified cause of waterborne outbreaks in the USA. *Giardia* cysts are more resistant than enteric bacteria to oxidative disinfectants such as chlorine, but they are not as resistant as *Cryptosporidium* oocysts. The time required for 90% inactivation at a free chlorine residual of 1 mg/l is about 25–30 minutes (WHO, 2011).

*Cryptosporidium* oocysts are found less frequently in groundwater than in surface water, although new data contradict previous assumptions that groundwater is inherently free of parasites such as *Cryptosporidium*. For example, Hancock *et al.* (1998) reported a study of 199 ground water samples tested for *Cryptosporidium*. They found that 5% of vertical wells, 20% of springs, 50% of infiltration galleries, and 45% of horizontal wells tested contained *Cryptosporidium* oocysts.

A number of cryptosporidiosis outbreaks have been associated with drinking water (Rose *et al.,* 1997; Solo-Gabriele and Meumeister, 1996). Deficiencies in water treatment systems are often cited as a major reason for outbreaks, and even the best of systems can be overwhelmed by a high density of oocysts entering the source waters over a short period of time.

Some investigators have given a general guide regarding *Cryptosporidium* levels and suggest that when estimating likely concentrations of *Cryptosporidium* in water, less than 0.1 oocyst/L may be assumed for pristine watersheds and boreholes and between 1 and 100 oocysts/L assumed for contaminated water, dependent upon catchment characteristics (Smith *et al*., 1995). Overseas investigations have shown that seasonal factors including run-off of land drainage may affect oocyst concentration by 10 fold with concentrations in drier periods being significantly lower than those in wetter periods.

# 3. MATERIALS AND METHODS

## 3.1. Description of the Study Area

The study was conducted at Inseno town, which is found in eastern part of Gurage zone in Southern Nations and Nationalities Region (SNNRP) of Ethiopia. The area is also bordering Oromiya Region in the north and east. Geographically, (Figure 1) located at 8.06ºN latitude and 38.47ºE longitude; and at a distance of 154 km from Addis Ababa, the capital city of the country. The town currently has a total population of 27500. Agro climatically, the area is situated in dry and tropical rainy /dry woinadega/ climatic zone which constitutes about 100% of the total area. Its elevation is 1842.5 meters above sea level (ITWSA, 2006EC).

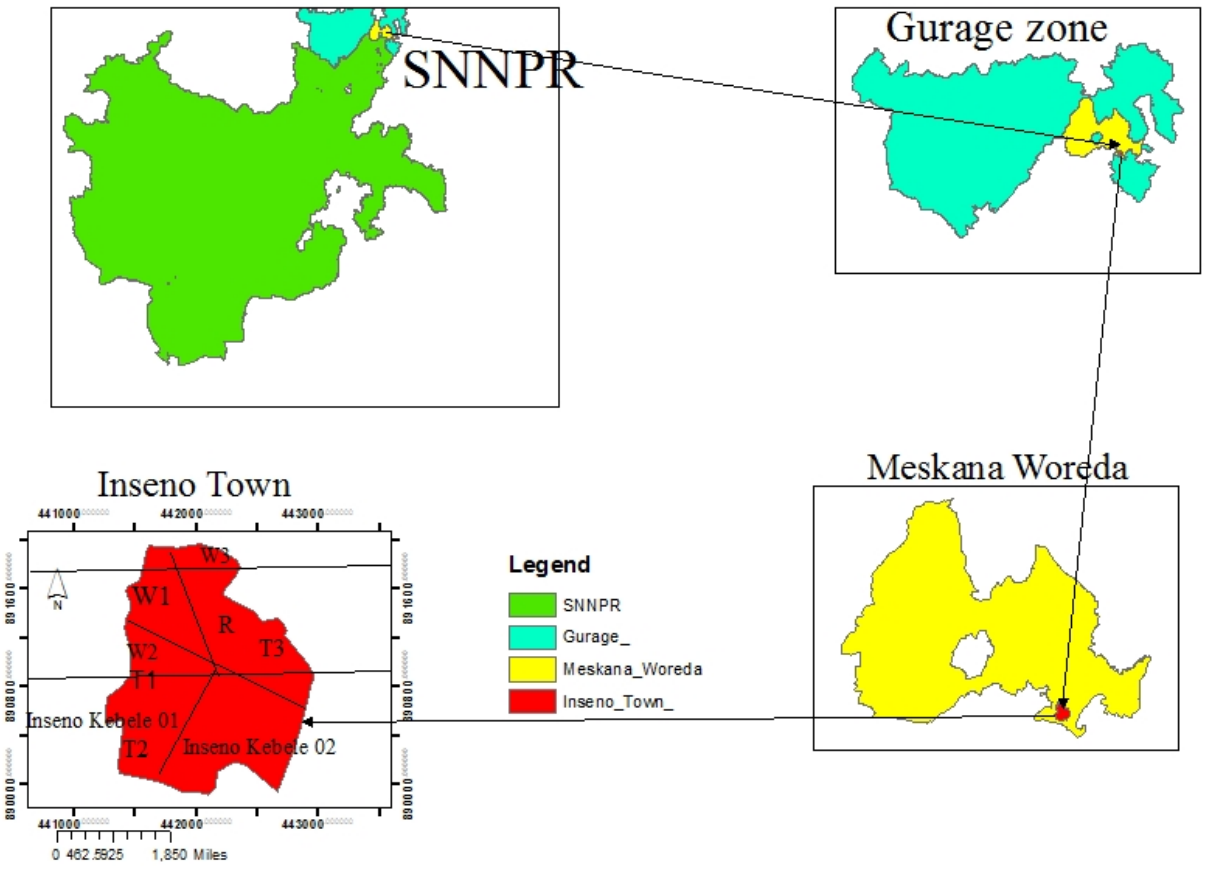


Figure 1: Map of the study area (source:Arc GIS)

## 3.2. Study Design

The study involved a laboratory based cross-sectional survey design. The design was intended to assess the physico-chemical and microbiological quality of groundwater that serves the community of Inseno town.

## 3.3. Data Type and Sources

Only primary data were used in this study. These data were mainly generated from laboratory experiments using water samples collected from ground water: reservoir, well and taps.

## 3.4. Sampling Points and Sampling Strategy

In this study, a total of seven sampling points were selected randomly to collect water samples. These sampling sites were constituted: one reservoir(R), three water wells (i.e., $W_1$, the well found next to Hibret Fire Elementary School in kebele 01;$W_2$, a second well found near to MuluWongel Church in kebele 01; $W_3$, a third well located next to the mosque in *kebele* 02) and tap water samples from pipes of three zones [(i.e. north zone pipe found next to Balewold Church and serving the areas surrounding *kebele* 01 ($Z_1$), west zone water pipe found next to MuluWongel Church and serving the area in *kebele* 01 ($Z_3$), and east zone water pipe serving the area around the water supply office ($Z_2$)].

The three distribution points were selected to represent the contamination profile of the groundwater in its course. Samples from the three wells (i.e., W1-W3) were selected to represent the contamination status of water at the level of the well before the water is pumped into the main reservoir. Therefore, the first activity in this study was to compare the level of contaminants of the three water samples obtained from three different wells.

Contaminants can get into the water from the reservoir due to poor handling of the reservoir. In order to determine the level of contamination, water samples were also collected from the reservoir and tested for microbial counts. This was the second activity. One reservoir was selected to assess the degree of contamination at the level where the water is temporarily stored.

Contaminants can also get into the water from the distribution lines due to leakages and rusts of water pipes. The third activity was, therefore, to determine the extent to which the water had been contaminated with microbes in the distribution lines (pipes). To this effect, water samples were collected from taps (distribution points) of the three selected distribution zones (i.e., Z1-Z3).

## 3.5. Water Sample Collection and Preparation

The water samples were collected once every week for a period of one month from each sampling point. Twenty eight (28) water samples were collected from the seven sampling sources i.e. from three wells, one reservoir and three taps. 300ml of water samples from each sampling points were collected in bottles for different bacteriological, physico-chemical characteristics and parasitological test. To collect samples for microbiological quality analysis, bottles were washed and rinsed with distilled water and sterilized in autoclave at $121^0$C for 15 minutes.

After sampling, bottles were kept in ice box and transported to Hawassa university medical microbiology laboratory, The bacteriological tests were undertaken within 6 hours after collection to avoid the growth or death of microorganisms in the sample (WHO, 2006). For floride and hardenss test, water samples were collected in 100 ml glass bottles, labeled and transported to the Hawassa university Environmental engineering laboratory in an ice box.

## 3.6. Sample Analysis

### 3.6.1. Bacteriological analysis of water samples

Bacteriological analysis was carried out for indicator organisms i.e. fecal coliform (*E.coli*) by most probable number (MPN) method. MPN protocol involves the primary presumption for the presence of coliform bacteria in the samples demonstrated by the appearance of gas in the lactose fermentation broth. For the presumptive test procedure 15 sets of test tubes containing

lactose fermentation broth required for each sample under analysis. Each test tube contained 10 ml of fermentation broth and inoculated with the water sample in asequential order of 10 ml in five of each 2X (double strength) lactose fermentation broth, 1ml in five of each 1x (single strength) lactose fermentation broth and lastly 0.1 ml in five of each 10 ml 1X (single strength) lactose fermentation broth.All the test tubes were incorporated with Derhum tubes for detection of gas formation by Gram negative coliform bacteria. Test tubes were incubated at 37$^{o}$C for 48 hours. After incubation, the number of tubes in which lactose fermentation with acid and gas production has occurred was counted. Finally, by referring to probability table (Macrady table) the MPN of coliform in 100 ml water sample was been estimated (Cheesbrough, 2006).

### 3.7. Parasitological Laboratory Examination Procedures

In order to undertake parasitological analysis for the presence of protozoan parasites, *Giardia, Entamoeba histolytica* and *Cryptosporidium parvum*, water samples were concentrated according to WHO (1991). Samples were transferred into 15ml of centrifuge tube and sedimented at 5000 RPM on centrifuge at 4$^{o}$C for 15 minutes. The sediments were preserved at 4$^{o}$C. The parasitological water quality (occurrence of *Giardia lamblia cyst, Cryptosporidium parvum oocyst, Entamoeba histolytica cyst)* was analyzed based on USEPA (USEPA, 2005).

#### 3.7.1 Direct wet smear

Using an applicator (wire loop), a small portion of 2-3 cm diameter of the preserved sediment was taken on clean slide. The sedments was spread over an area of approximately 2cm x1cm and the sediments were covered with a cover slip. Finally, the cysts were examined under the microscope using 10-40x objectives (WHO, 1991). The cyst was used to identify following the procedure of WHO parasitological laboratory examination (WHO, 1991).

### 3.7.2 Modified Ziehl-Nelson method

Aportion of the sediment was emulsified on clean slide and spread over an area of 2cmx 1cm. The resulting smear was then being allowed to dry and be fixed using absolute methanol for 10 minutes. After fixing, the slide was flooded with carbol fuchsine for 20 minutes and eventually rinsed in tap water for 20 minutes. It is then decolorized in 3% acid alchol for 20 minutes, rinsed with tap water for additional 20 minutes and finally flooded with 0.3% methylene blue. The presence of oocyst has examined microscopically under oil immersion objective (WHO, 1991).

## 3.8. Physico-Chemical Analysis

The main physico-chemical parameters like temperature, pH, turbidity and free chlorine were measured directly on the site of sample collection due to their unstable nature. Thermometer calibrated from 0 $^{o}$C to 100 $^{o}$C was used for temperature measurments. The pH of the water sample was measured with a pH meter. Turbidity meter (model, wag-wt3020ki) was used to determine turbidity of the water sample. Free chlorine residues for each sample was determined at the site of collection with a comparator system using a DPD-1chlorine tablet.

### 3.8.1. Estimation of total hardness of water

100ml of given water sample was pipette out in a washed conical flask. 1ml of ammonia solution (concentration of ammonia) and 2-3 drops of Eriochrome Black-T indicator was added, the colour of the solution turns wine red. This solution was titrated against EDTA solution taken in the burette was noted and the titration repeated to get concordant value. Finally using the analytical calculation, (the volum of titrant EDTA multiplied by one thousand over one handred(the sample volume) total hardness of given water sample was determined by

calculation and compared with water quality guide lines. Fluorides was determined using DR2800 Spectrophotometer machine (model, 7100).

## 3.9. Ethical Consideration

Data collection was conducted after obtaining informed consent from the concerned office such as Inseno Town Water Service Authority (ETWSA). With regards to data collection at the selected points, the study objectives were clearly explained to the responsible persons. The responsible persons was assured that the information given to them used only for the purpose of the research.

## 3.10. Data Analysis

Data was organized and summarized using software SPSS appropriate methods. Analysis was done using a simple descriptive statistics assisted by Microsoft excel. Analyses of variance (ANOVA) at 5% level of significance were used to compare the quality of water samples of different sources. The results were analyzed using statistical software SPSS version 20. The microbiological quality of water were compared with standards of who guidelines for drinking water quality and considered as acceptable or unacceptable (WHO, 2011).

# 4. RESULTS AND DISCUSSION

## 4.1.Physico-Chemical and Bacteriological Water Quality Analyses

### 4.1.1.Bacteriological analysis of well water samples

Twelve (12) well water samples were collected from the study area. All the water sources had no chlorine treatment. From these water sources 50% presumptive bacteria showed above the presumptive limit for drinking water (Table1). This could be due to the presence of some wells that were near to latrine and some were without cover. From the total sample 50% of presumptive bacteria count MPN within acceptable coliform counts (less than 10 MPN per

100ml of water). In relation to distance of water sources from latrine, wells nearest to Hibret Fire Elementary School in kebele 01 of water sources found at a distance of less than 30m which is below WHO recommendation for minimum distance that should be exist between latrine and water source. On top of this wells (W2) next to MuluWongel Church of water source were without cover. Because of that reason, water sample that taken from that well was contaminated with bacteria

In this study 50% of well water sample have MPN of colony above the allowable limit. This indicates that almost half of the water sources of town were fecally polluted. Supply of water that owes no threat to the consumer's health depends on continuous protection because of human frailty associated with protection, priority should be given to selection of the purest source. Polluted sources should not be used unless other sources are economically unavailable. Frequent examination of fecal indicator organisms remain the most sensitive way of assessing the hygienic conditions of water (World Health Organization, 2003). In comparison with study conducted in Uganda, in 2002, it showed that 90% samples had exceeded the WHO guideline (Haruna *et al*., 2005). If we compare the finding of this study with a study conducted in Jimma town in 2005, it showed that 95.8% of samples were unacceptable or grossly contaminated.This difference in percentage might be due to the appropriate location of wells with respect to latrine or very low environmental protection. In our study, from total analyzed twelve water samples, there were 6 water samples with MPN less than 10 per 100ml of water, from those four of them were from unprotected well and two of them from protected well. According to research conducted south western Saudi Arabia (Alotaibi, 2009) and in Tamil Nadu (Rajendram *et al*., 2006) all well water sources were positive for coliforms using MPN method whereas in our study, one well was free of coliform. The gap might be due to the

protection of wells, the appropriate location of wells with respect to latrine. Wells should be placed at higher place than latrins (Ethiopian federal MOH, 2004).

Table 1: MPN index value for various combinations of positive results for well water samples.

| Site | Sample | Number of tubes giving a positive reaction from | | | |
|---|---|---|---|---|---|
| | | 5 of 10 ml each | 5 of 1 ml each | 5 of 0.1 ml each | MPN/100 ml |
| W1 | S1 | 0 | 1 | 0 | 2 |
| | S2 | 0 | 0 | 0 | <2 |
| | S3 | 0 | 0 | 0 | <2 |
| | S4 | 0 | 0 | 0 | <2 |
| W2 | S1 | 4 | 1 | 0 | 17 |
| | S2 | 3 | 2 | 1 | 17 |
| | S3 | 3 | 1 | 1 | 14 |
| | S4 | 4 | 0 | 1 | 17 |
| W3 | S1 | 1 | 1 | 0 | 4 |
| | S2 | 1 | 0 | 1 | 4 |
| | S3 | 4 | 0 | 0 | 13 |
| | S4 | 4 | 0 | 1 | 17 |

**Key:** W=well, S=sample

### 4.1.2 Bacteriological analysis of tap water samples

Twelve tap water samples were collected from the study area. From these tap water 8.3 % presumptive bacteria count MPN above the presumptive limits for drinking water (Table 2). The minimum percent (8.3 %) of colony at Tap2 presumptive bacteria count MPN above the presumptive limits for drinking water may have been because of re-growth of microorganisms in bio-films, which are formed on interior surfaces of water pipes. From the total sample 91.7% of presumptive bacteria count MPN within acceptable coliform counts (less than 2 MPN per 100ml of water).

In relation to distance of water sources from latrine, all of the water sources found at a distance of above 30m which is within WHO recommendation for minimum distance that should exist between latrine and water source (Table 2). The study conducted in Peshawar, 2006 (Hamida *et al*.,2006) reported that hand pump drinking water of 92 % samples were unfit for drinking due to presence of high bacterial load and pathogenic bacteria.

### 4.1.3 Bacteriological analysis of reservoir water samples

Four reservoir water samples were collected from the study area. From these reservoir water samples 100 % of presumptive bacteria count MPN were within the presumptive limits of WHO for drinking water (less than 2 MPN per 100ml of water) as shown in Table 2. In relation to distance of reservoir from latrine, the reservoir found at a distance of above 30m which is within WHO recommendation for minimum distance that should be exist between latrine and water source. The similar study conducted at Nepalese (Pradhan, 2016 ) reported that 22.0% of over head tank, 30% of piped tap water, 40% of natural tap and non of the house hold filter water showed fecal coliform contaminaton. The finding of this study was higher as compaired with the present study. The similar study conducted at western

Maharastra (Tambe *et al*., 2008) reported that 49.8% of the 313 samples which were collected from wells, tank, community stand post, hand pumps, percolation lakes, and streams, and from households were polluted, and water from piped supply showed a pollution rate of 45.9%.

Table 2: MPN index value for various combinations of positive results tap water and reservoir samples

| Site | Sample | Number of tubes giving a positive reaction from | | | MPN/100ml |
|---|---|---|---|---|---|
| | | 5 of 10 ml each | 5 of 1 ml each | 5 of 0.1 ml each | |
| T1 | 1 | 0 | 0 | 0 | <2 |
| | 2 | 0 | 0 | 0 | <2 |
| | 3 | 0 | 0 | 0 | <2 |
| | 4 | 0 | 0 | 0 | <2 |
| T2 | 1 | 0 | 0 | 1 | 2 |
| | 2 | 0 | 0 | 0 | <2 |
| | 3 | 0 | 0 | 0 | <2 |
| | 4 | 0 | 0 | 0 | <2 |
| T3 | 1 | 0 | 0 | 0 | <2 |
| | 2 | 0 | 0 | 0 | <2 |
| | 3 | 0 | 0 | 0 | <2 |
| | 4 | 0 | 0 | 0 | <2 |
| R | 1 | 0 | 0 | 0 | <2 |
| | 1 | 0 | 0 | 0 | <2 |
| | 1 | 0 | 0 | 0 | <2 |
| | 1 | 0 | 0 | 0 | <2 |

**Key**: T= tap, R= reservior,MPN=most probable number

### 4.1.4. Physico-chemical analysis of well water

Temperature is one of the physico-chemical parameters used to evaluate water quality of potable water**.** The data showed that the highest temperature of 23.56$^{o}$C was measured from Well water 3 (W3), whereas the lowest mean record of 22.98$^{o}$c measured from Well 2 (W2). However, the water samples from the three wells (water sources) did not show significant difference amongst one another (Table 3).

A similar study conducted in Ziway, a town located near the study area, showed a mean temperature of 23.2$^{o}$C from different water source samples (Kassahun, 2008), whereas a mean temperature record of 23.8$^{o}$C was measured from drinking water source supply of Bahirdar town (Getnet, 2008). And also a mean temperature record of 24.4$^{o}$C was measured from drinking water source supply of Debrezeit (Bishoftu) town (Desta, 2009) while a slightly higher temperature of 25.5$^{o}$C was reported from water source samples from Nigeria (Agbogu *et al*, 2006). All the above mentioned were characterized by high temperature and this factor may have contributed to the high temperature records of water samples that did not meet the WHO standard of <15$^{o}$C (WHO, 1997).

Table 3. Mean values (±SD) of physico-chemical parameters of well water samples

| **Parameter** | **Sampling sites** | | | |
|---|---|---|---|---|
| | W1 | W2 | W3 | WHO |
| Temp ($^{o}$C) | 23.2$^{a}$±0.17 | 22.98 $^{a}$ ±2.03 | 23.56 $^{a}$ ±1.4 | <15$^{o}$C |
| pH | 6.72 $^{b}$ ±0.4 | 7.22 $^{a}$±0.17 | 7.3 $^{a}$±0.18 | 6.5-8.5 |

| | | | | |
|---|---|---|---|---|
| Turbidity (NTU) | 5.73 $^{b}\pm$3.4 | 22.3 $^{a}\pm$12.87 | 17.7$^{ab}$ ±4.1 | 5NTU |
| Fluoride (mg/l) | 1.22 $^{a}$ ±0.27 | 1.24 $^{a}\pm$0.19 | 0.87 $^{b}\pm$0.03 | <1.5 mg/l |
| Hardness (mg/l) | 120.75 $^{a}\pm$55.49 | 199.75$^{a}\pm$18 | 130$^{a}\pm$44.26 | <500 mg/l |

Means with the same letter are not significantly different within the row.

**Key:** Temp=temperature, FCR=residual free chlorine, W= well water, NTU=Nephelometric turbidity unit

With regard to pH test of well water sample, it was found between pH 6.72 recorded from well 1(W1) and pH 7.3 recorded from well 3 (W3). It did show significant difference in pH,) (Table 3). In general, the overall pH records of water samples from the sources were found to be slightly basic. These records are significantly different from the mean pH value of 6.2 recorded from the same water sources analyzed in 1990 during the time of exploration and extraction (WSSA, 1990). However, the finding of the present study is comparable to the average basic pH record of 7.6 from water sources at Akaki-Kaliti sub-city of Addis Ababa (Mengstayehu, 2007), a record of pH 8.3 from water sources at Ziway town (Kassahun, 2008), and measurement of pH of 7.8 from Adama (Nazareth) town (Temesgen, 2009). Generally, the pH status of Inseno water walls was within the recommended standard limits of 6.5-8.5 (WHO, 1997).

Turbidity of water is one of important physical parameters that affect not only the quality of water, but also other chemical and bacteriological parameters and efficiency of the treatment of water (WHO, 2006). The highest and lowest turbidity measurements were recorded from water samples of Well 2 (W2) and Well 1(W1) with 22.3 NTU and 5.75 NTU, respectively (Table 3). The measurements showed significant variations among water samples of W1and W2. In general, the average turbidity values of wells (W1-W3) (15.26 NTU) were found to be

significantly higher than the average NTU of 2.3 recorded at Debrezeit (Bishoftu) town (Desta, 2009)**.** The finding of this particular study showed that, the turbidity level of all source water samples were above the WHO standards (≥5NTU) (WHO, 1997). Since turbidity is the measure of cloudiness of water, it can indicate that pathogens may contaminated the water (Olson, 2004).

Fluoride concentration is an important aspect of hydrogen chemistry, because of its impact on human health. The overall mean value analyzed for all water sample sources were ranged between 0.87-1.24 mg/l in this study area. The recommended concentration of fluoride in drinking water is less than 1.5 mg/l (WHO, 2006). Low fluoride content less than 0.6 mg/l causes dental caries, whereas high content greater than 1.2 mg/l fluoride levels results in fluorosis. Hence, it is essential to have a safe limit of fluoride concentration between 0.6 and 1.2 mg/l in drinking water (Jitendra *et al.*, 2008). High concentration of fluoride can cause dental and skeletal fluorosis such as mottling of teeth, deformation of ligaments and bending of spinal cord (Janardhana Raju *et al.*, 2009). Therefore, the fluoride content value observed in this study area exceed the WHO (2006) guide line value less than 1.5 mg/l.

Total hardness is very important parameter in decreasing the toxic effect of poisonous element. As shown in Table 2, the mean value of hardness was found to be in the range of 199.75 to 120.75mg/l in all water samples. This value is with in the medium hardness of water level. All samples investigated in this study area did not exceed the limits of 500 mg/l of WHO (2006) standards.

### 4.1.5. Physico-chemical parameters of tap water samples

Apart from the water sources, it is all the more important to analyze the water quality of the tap water distribution system that reaches the households and determines the health status of the population Table 4 shows the similarities and differences of the tap water samples of the different locations of the town with regard to the tested physico-chemical parameters.

Table 4: Mean values (±SD) of physico-chemical parameters of tap water samples

| **Parameter** | **Sampling sites** | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | WHO |
| Temp( $^{o}$C) | 23.65 $^{b}$ ±0.45 | 23.3 $^{b}$ ±1.46 | 22.13 $^{b}$ ±1.55 | <15$^{o}$C |
| pH | 6.69$^{a}$ ±0.3 | 6.83 $^{a}$±0.23 | 6.42 $^{a}$±0.09 | 6.5-8.5 |
| Turb (NTU) | 0.93 $^{a}$ ±0.25 | 2.14 $^{a}$ ±0.97 | 1.3$^{ab}$ ±0.68 | 5NTU |
| FCR(mg/l) | 0.26 $^{b}$ ±0.16 | 0.32 $^{b}$ ±0.26 | 0.12 $^{b}$ ±0.06 | 0.2-0.5 |
| Fluorid(mg/l) | 0.35$^{a}$ ±0.28 | 1.03$^{b}$±0.14 | 1.1$^{b}$±0.11 | <1.5 mg/l |
| Hardnes(mg/l) | 86 $^{b}$ ±25.01 | 165.75 $^{b}$ ±56.5 | 117 $^{b}$ ±64.31 | <500 mg/l |

Means with different letter are significantly different within the row

**K**ey: Temp=temperature, FCR=residual free chlorine, T=tap, Turb=turbidity,NTU=Nephelometric turbidity unit

With regard to temperature, the highest and lowest values were recorded from water samples of Tap1(T1)and Tap 3(T3) with 23.65$^{o}$C and 22.13$^{o}$C respectively (Table 4). Likewise, the pH of tap water samples fell between pH 6.42 (Tap3) and pH 6.83 (Tap2) (Table 4). The pH range of tap water samples is lower than the pH range of well water samples. This may have

been because of acidity of the water .The pH measurement of tap water systems of the present study fell between the pH records of (6.42-6.83) the same study conducted at both Ziway town (Kassahun, 2008) and Akakikalit sub city of Addis Ababa (Mengstayehu, 2007) tap water samples.

The turbidity measurements of the tap water samples did not show significant difference between sampling sites. The highest turbidity of 2.14 NTU was measured at sampling site of Tap 2 (T2) (Table4); whereas, the lowest turbidity of 0.93 NTU was recorded from Tap1 (T1). The turbidity record of the present study (0.93-2.14NTU) was slightly lower than the lowest limit and the highest limit of 1.6-4.4 NTU from Bahir Dar town (Getnwt, 2008) and slightly higher than the lowest limit. But it is slightly lower than the highest limit of 0.1-5.0NTU from Akakikalit sub city of Addis Ababa (Mengstayehu, 2007) tap water samples, respectively.

As far as the free-residual chlorine (FRC) contents in the tap water system are concerned, the highest concentration was recorded from tap water samples of Tap 2 (T2) (0.32mg/l), and the lowest concentration was recorded from tap water samples of tap 3 (T3) (0.12mg/l). About 75% of the samples from tap 3 (T3) and 25% of each of tap1 and tap2 contain FRC below the treatment standard using chlorin set by WHO (1997). All taken together about 85 % of tap water samples were found to be with in the recommended limits of 0.2-0.5mg/l of FCR in tap water treatment standard set by WHO (1997) and NGL (2002). Similarly, (Kassahun, 2008) reported 37.5 % and 97.7% of tap water samples were below the recommended limits of 0.2-0.5 mg/l of FCR in tap water distribution systems from Ziway and Bahir Dar towns respectively. In general this all indicates that there is poor water chlorination mechanism in different towns in the country.

Fluoride concentration has an impact on human health. The overall value mean value analyzed for all water sample sources were ranged between 1.1 to 0.35 mg/l in this study area. The recommended fluoride concentration of floured in drinking water is less than 1.5 mg/l (WHO, 2006). Therefore, the fluoride content value observed in this study area in agreement with the WHO (2006) guide line value less than 1.5 mg/l.

Total hardness is very important parameter in decreasing the toxic effect of poisonous elements. As shown in table: 4 the mean value of hardness was found to be in the range of (86 to 165.75mg/l) in all water sample. This value is with in the medium hardness of water level. All samples investigated in this study area not exceed the limits of 500mg/l of WHO (2006) standards.

**4.1.6.Physico-chemical parameters of reservoir water samples**

Apart from the water sources, water quality of Enseno town water was monitored as it moved from source to the distribution system. Table 5 shows the results of the different tests on the reservoir. Accordingly, the mean temperature records of the water samples of the distribution network system were 24.8$^{o}$C.

Table 5: Mean value (±SD) of Physic-Chemical Analysis of reservoir water samples

| Parameter | Sampling sites |
|---|---|
| | R |
| Temp. ( $^{o}$C) | 24.8±0.45 |
| pH | 6.36±0.23 |
| Turb (NTU) | 0.7±0.65 |
| FCR (mg/l) | 0.78±0.93 |

| | |
|---|---|
| Fluoride (mg/l) | 0.81±0.51 |
| Hardness( mg/l) | 226.75±23.92 |

**Key**: R=reservoir, Turb=turbidity, Temp=temperature, FCR=free residual chlorine

The mean pH measurements of distribution system (reservoir) were found to be pH 6.36 (Table 5). The pH measurement of the reservoir was slightly lower than the water sources (well water (pH 7.3 – pH 6.72) and tap water (pH 6.42 – pH 6.83). It may well be that microbial load of the water samples may influence the pH difference among well water, the tap water and reservoir water that was either the mineral concentration of the soil or environmental condition.

As far as the mean turbidity of the water samples from the reservoir is concerned, the data showed turbidity measurement of 0.7 NTU. It is interesting to note that, the reservoir water showed a slight decrease (average 0.7NTU) in turbidity compared to the well water samples of 15.26 NTU. Slightly higher mean turbidity of reservoir water samples of 0.9NTU were conducted at Welkite town (Dessalew, 2018).

The mean free residual chlorine (FCR) of the distribution system to be 0.78. The reservoir water showed higher increase (average 0.78 mg/l) in free chlorine residual compared to the source (the well water) of 0.0 mg/l. The mean value of the samples were above the guideline value of 0.2-0.5 of residual chlorine in the water distribution system.

The overall fluoride concentration value mean analyzed for all water sample of reservoir was (0.81mg/l) in this study area. The recommended fluoride concentration in drinking water is less than 1.5 mg/l (WHO, 2006). Therefore, the fluoride content value observed in this study area in agreement with the WHO (2006) guide line value less than 1.5 mg/l. The mean value of hardness was found to be 226.75 in all water sample. This value is within the recommended

limit hardness of water level. All samples investigated in this study area not exceed the limits of 500mg/l of WHO (2006) standards.

## 4.2. Parasitological Quality of Drinking Water Sources

The parasitological analysis of water sources in (Table 6) showed that reservoir, well and tap water were negative for *amoeba cyst*, *cryptosporidium,* whereas about 8.3% of well water had *Giardia*. This was because of either the giardia enter in to the well during the wind time with dust or during rainy time the giardia enter the well with erosion. They may be eroded with soil. 91.7% of well water were free from Giardia in (table 7). Whereas about 100% of water samples were negative with *E. histolytica* and *Cryptosporidium oocysts* respectively.

Similar study conducted in Addis Ababa drinking water sources demonstrated that, there was significant difference in concentration of Giardia and cryptosporidium between treated and untreated water (Nigus *et al*., 2008). For instance, ground water is usually free of Giardia Amoeba and Cryptosporidium but it can be contaminated occasionally from surface activities through infiltration (Lechevallier *et al*., 1995). An investigation made by (Stoyanovai *et al*. ,2006) on drinking water supply contamination with *Giardia, Amoeba and cryptosporidium* in Varma found positive with an average number of 5 cysts or occyst⁄ liter. This differences may be compared to the present finding resulted due to the source of contaminations, lack of adequate water treatment and unhygienic practices near and around the water sources and distribution line systems. Protection of water sources and treatment of water supplies have greatly reduced the microbial loads especially the protozoan parasites in water sources (WHO, 2003).

Table 6: Parasitological analysis of three types of water sources

| Water source | *Giardia lamblia* | *Entamoeba histolytica* | *Cryptosporidium parvum* |
|---|---|---|---|
| Tap water | -ve | -ve | -ve |
| Well water | +ve | -ve | -ve |
| Reservoir water | -ve | -ve | -ve |
| % of parasite in water sample | 3.57 % | 0 % | 0 % |
| Total | 100% | 100% | 100% |

**Key: -**ve negative, +ve positive

Similarly, (Hancock *et al*., 1997) collected 463 ground water samples from 199 sites and found Giardia cysts in 1% of the vertical wells and 36% of the horizontal wells. Hiber (1988) also found *Giardia cysts* in only 19% of tube wells and 3% of wells sampled. On the country, Archer *et al*., (1995) did not detect *Giardia* in any of the 17 samples they collected from six wells in Wiscosin. Study conducted at Leon Nicaragua (Leiva *et al*., 2008) and at Bulgaria (Tsvetkova *et al*., 2004) reported that the *amoebae* were found in 43% and 61% respectively of the water samples.

## 4.3 Correlation Matrix

The selected water quality parameters were analyzed to determine one parameter could be used as an indicator for the other variables .some of the physic-chemical parameters were found to be negatively correlated with each other. For instance Temprature negatively related

significantly with fluoride (r=-0.938) and (r=-0.522) for reservoir and well waters respectively (Table 8, 10)

From (table 8 and 10) below Residual chlorine had negative relationship with other physico-chemical parameters in reservoir and tap water samples. This shows that as residual chlorin concentration decreases other variables such as pH, turbidity, and temperature increasing in drinking water. Similarly the study conducted at metropolis, Ghana by Karikari and Ampoto (2013) showed that there was a significant positive correlation between pH and turbidity (r=0.79).

Also the temperature of reservoir water and tap water had negative correlation with all parameters except pH, residual chlorine, hardness in tap water which was positively correlated in tap water while temperature was positively correlated with all parameters in well water excluding PH and fluoride. This analysis viewed that warm conditions can activate the re-growth of microorganisms in the distribution systems (Ngclcani, 1998).similar kind of research in Italy showed that the survival curves of Aeromonas decline rapidly at low temperatures (5$^{O}$c).where as survival at temperature greater than 20$^{O}$c increases (Sisti *et al.,* 1998)

Table 8: Correlation of physico-chemical parameters of reservoir water: Correlation of physico-chemical parameters of reservoir water

| | Temp | pH | Turbidity | Chlorine | Fluoride | Hardness |
|---|---|---|---|---|---|---|
| Temp | 1 | | | | | |
| pH | -0.801 | 1 | | | | |
| Turbidity | -0.338 | 0.086 | 1 | | | |
| Chlorine | -0.710 | 0.707 | -0.423 | 1 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Fluoride | -0.938 | 0.822 | -0.010 | 0.911 | 1 | |
| Hardness | -0.659 | 0.673 | -0.485 | 0.998** | 0.879 | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9: Correlation of physico-chemical parameters of tap water

| | Temp | pH | Turbidity | Chlorine | Fluoride | Hardness |
|---|---|---|---|---|---|---|
| Temp | 1 | | | | | |
| pH | 0.110 | 1 | | | | |
| Turbidity | -0.017 | 0.508 | 1 | | | |
| Chlorine | 0.370 | -0.229 | -0.239 | 1 | | |
| Fluoride | -0.276 | -0.263 | 0.459 | -0.009 | 1 | |
| Hardness | 0.062 | 0.425 | 0.759** | -0.232 | 0.368 | 1 |

**. Correlation is significant at the 0.01 level (2-tailed).

Table 10: Correlation of physico-chemical parameters of well water

| | Temp | pH | Turbidity | Chlorine | Fluoride | Hardness |
|---|---|---|---|---|---|---|
| Temp | 1 | | | | | |
| pH | -0.428 | 1 | | | | |
| Turbidity | 0.412 | 0.100 | 1 | | | |
| Chlorine | .a | .a | .a | 1 | | |
| Fluoride | -0.522 | 0.141 | -0.608* | .a | 1 | |
| Hardness | 0.071 | 0.315 | 0.474 | .a | -0.368 | 1 |

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because the well was not treated by chlorine.

# 5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 5.1. Summary and Conclusions

There is a global concern about pollution of water bodies worldwide with their ultimate impact on the natural ecosystem as well as human health. It was for a reason of such concern that the present study was conducted by selectively analyzing parameters relevant for indicating pollution of groundwater and the quality of water in the reservoir, taps and walls used for human consumption in Inseno town

In this study, water samples were collected from wells, distribution points and reservoir and tested in the laboratory for the presence of bacteriological coliforms and detection of *Giardia* Spp., *Cryptosporidium, E.histolytica* Spp.The basic bacteriological methods used in this study were most probable number method and concentration method for *Giardia*, *Cryptosporidium* and *E.histolytica* analysis

Bacteriological quality of some drinking water samples analyzed in this study did not meet the standards set for drinking water by WHO (2006). All water samples that were taken from reservoir met the standards set for drinking water by WHO (2006) that means <2MPN per 100ml. In the other case almost 50% of wells water samples have MPN of colony above the allowable limit and 50% of well water samples have MPN of colony within the allowable limit. In case of tap water samples, 8.3 % were presumptive bacteria count MPN above the presumptive limits for drinking water and about 91.7% presumptive bacteria count MPN of colony with in allowable limit.

Parasitological quality of drinking water samples analyzed only about 3.57% of water samples were positive with Giardia cyst and 96.43 % of all water samples free from *Giardia cyst. E. histolytica* and *Cryptosporidium* were absent in all drinking water samples.In this study the

physico-chemical quality of the well water systems of Inseno town met both the WHO and national standards except temperature and turbidity. In case of tap water system all water samples met WHO and national standards except for temperature and pH. The temperature records from wells to tap water samples showed a higher measurement (22.13 $^{o}$C-24.8$^{o}$C) compared to the standard of <15$^{o}$C.About 15% of tap water samples analyzed showed FCR values less than the WHO values of 0.2-0.5mg/l. This indicates there was insufficient chlorination in the treatment system. With regard to turbidity, 100% of the water samples from the reservoir and tap water were within acceptable limit of <5NTU.

## 5.2. Recommendations

The following recommendations were forwarded in view of the findings of this study.

For provision of potable water chlorination should be done frequently and regular monitoring of residual chlorine in the distribution system should be carried out to ensure that residual chlorine of 0.2-0.5 mg/l is available at the consumer end.

Immediate and proper maintenance should be employed when the water distribution lines were broken.

As almost half of well water samples had unacceptable bacterial count and all the well waters which were positive for presumptive coliform count and the coliform showing fecal contamination of well water and regular disinfection of drinking water sources, periodic bacteriological appraisal of drinking water sources and construction and distribution of piped water required.

The present work is limited to few physic-chemical parameters. Therefore, year round analysis of additional water quality parameters such as heavy metals, total dissolved solids and dissolved oxygen should be undertaken.

Finally, further operational research is needed to determine the seasonal variation on the contamination level of drinking water, to assess pathogen loads in water sources to develop risk-reducing water quality management systems.

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# 7. APPENDICES

## 7.1. Appendix A

appendix 1 Analysis of variance for physico-chemical parameters of well water samples

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| well temperature | Between Groups | .727 | 2 | .363 | .177 | .840 |
| | Within Groups | 18.442 | 9 | 2.049 | | |
| | Total | 19.169 | 11 | | | |
| well pH | Between Groups | .782 | 2 | .391 | 5.211 | .031 |
| | Within Groups | .675 | 9 | .075 | | |
| | Total | 1.457 | 11 | | | |
| well turbidity | Between Groups | 586.032 | 2 | 293.016 | 4.526 | .044 |
| | Within Groups | 582.634 | 9 | 64.737 | | |
| | Total | 1168.666 | 11 | | | |
| well chlorine | Between Groups | .000 | 2 | .000 | . | . |
| | Within Groups | .000 | 9 | .000 | | |
| | Total | .000 | 11 | | | |
| well flourid | Between Groups | .339 | 2 | .170 | 4.544 | .043 |
| | Within Groups | .336 | 9 | .037 | | |
| | Total | .675 | 11 | | | |
| well hardness | Between Groups | 14922.167 | 2 | 7461.083 | 4.172 | .052 |
| | Within Groups | 16095.500 | 9 | 1788.389 | | |
| | Total | 31017.667 | 11 | | | |

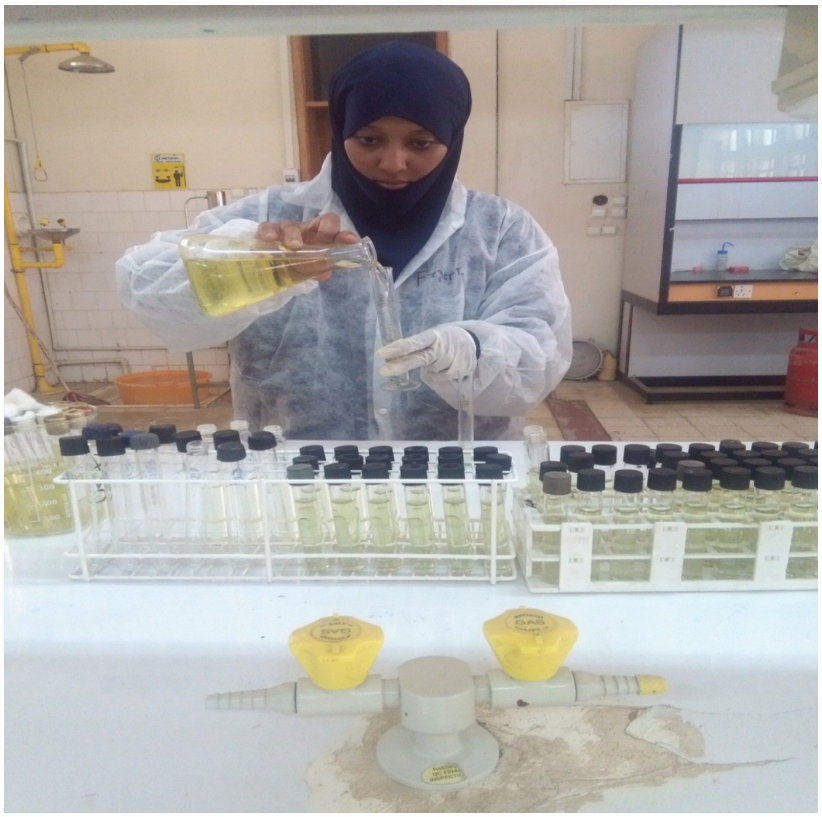
Appendix 2 Analysis of variance for physico-chemical parameters of tap water samples

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| tap water temperature | Between Groups | 5.040 | 2 | 2.520 | 1.580 | .258 |
| | Within Groups | 14.351 | 9 | 1.595 | | |
| | Total | 19.391 | 11 | | | |
| tap water PH | Between Groups | .347 | 2 | .173 | 3.321 | .083 |
| | Within Groups | .470 | 9 | .052 | | |
| | Total | .816 | 11 | | | |
| tap water turbidity | Between Groups | 3.082 | 2 | 1.541 | 3.119 | .094 |
| | Within Groups | 4.446 | 9 | .494 | | |
| | Total | 7.528 | 11 | | | |
| tap water chlorine | Between Groups | .086 | 2 | .043 | 1.282 | .324 |
| | Within Groups | .302 | 9 | .034 | | |
| | Total | .388 | 11 | | | |
| tap water fluoride | Between Groups | 1.379 | 2 | .689 | 18.328 | .001 |
| | Within Groups | .338 | 9 | .038 | | |
| | Total | 1.717 | 11 | | | |
| tap water hardness | Between Groups | 12930.167 | 2 | 6465.083 | 2.438 | .143 |
| | Within Groups | 23866.750 | 9 | 2651.861 | | |
| | Total | 36796.917 | 11 | | | |

*appendix 3 Analysis of variance for physico-chemical parameters of reservoir water samples*

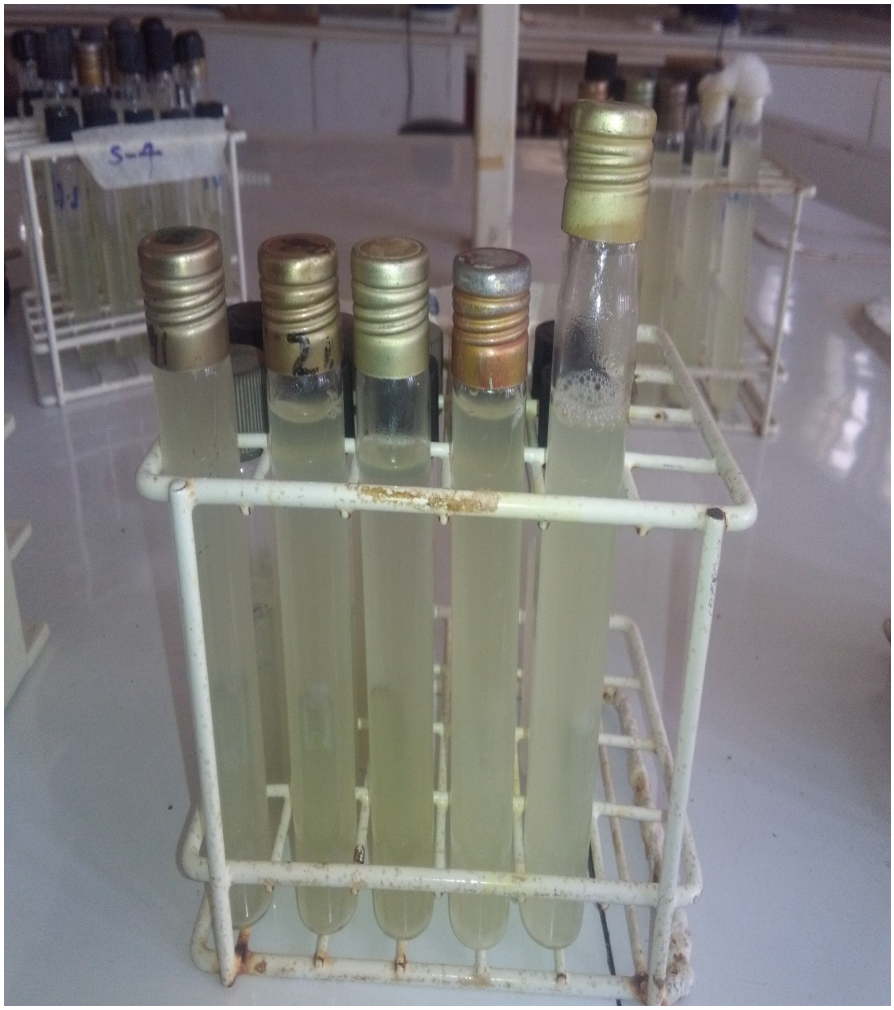
| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Temperature of reservoir water | Between Groups | .620 | 3 | .207 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | .620 | 3 | | | |
| pH of reservoir water | Between Groups | .163 | 3 | .054 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | .163 | 3 | | | |
| turbidity of reservoir water | Between Groups | 1.278 | 3 | .426 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 1.278 | 3 | | | |
| chlorin of reservoir water | Between Groups | 2.574 | 3 | .858 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 2.574 | 3 | | | |
| flourid of reservoir water | Between Groups | .787 | 3 | .262 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | .787 | 3 | | | |
| hardeness of reservoir water | Between Groups | 1716.750 | 3 | 572.250 | . | . |
| | Within Groups | .000 | 0 | . | | |
| | Total | 1716.750 | 3 | | | |

## 7.2. Appendix B



Appendix figure 1: Preparing media in the laboratory

Appendex figure 2: Collecting water samples from the study area




Appendex figure 3: Lactose broth (10 ml double strength) showing acid and gas production