1. INTRODUCTION

Water

Water is a vital material with unique physical and chemical properties. Life originated in water, is thriving in water, water being it's solvent and medium. It is the matrix of life. It is the integrated system of biological metabolic reactions in aqueous solution that is essential for the maintenance of life.

Safe drinking water is defined as water with microbial, chemical and physical characteristics that meet WHO guidelines or national standards on drinking water quality (WHO, 2007). Water being so important to all life, including man, the pollution of water is a threat to the survival and existence of life itself.

There is intense pressure on the water resources being used in Nepal due to the limited amount available with respect to demand over the past few decades, the population in the country has grown rapidly at over 2% per annum. Urbanization caused by natural growth and migration is another factor that puts pressure on the existing water supply in urban areas. Natural factors, such as landslides and floods, also put pressure on water resources by demanding reservoirs and irrigation canals. All these activities affect the quality of water (UNEP, 2001).

Much of the Nepalese population uses surface water for potable supply which is most vulnerable to pollution. Hence only 34% of the population is thought to have access to safe drinking water (Nepal Net, 2001). Use of ground water for drinking purpose is extensive in the Kathmandu Valley. About 46 percent of water supply in Kathmandu and Lalitpur is from underground source. Shallow ground waters are also at risk from contamination. Pathogenic bacteria, pesticides, nitrate and industrial effluents (urban and peri-urban areas) are likely to be the greatest problems encountered. Jacobson, (1996) reported shallow ground waters in the Kathmandu Valley in particular have been highly contaminated with industrial and domestic pollutants in recent years.

Water Sources

Earth's surface consists of 70% water. Water is available almost everywhere if proper methods are used to get it. Sources where water may be obtained include: ground sources (e.g. aquifers, aquitards, etc.), water from the sky (also called storm water; which includes rain, hail, snow, fog, etc.), surface water (e.g. streams/rivers), and other sources (such as plants, animals, etc.)

Water Sources in Kathmandu Valley

Water in the Kathmandu valley is derived from two sources: Surface water (rivers and ponds) and ground water. They are basically fed with rainfall (ICIMOD/MOEST/UNEP 2007).

Ground water: Ground water has remained to be a major water supply source for a population of 1.5 million at present in the valley. About 50% of the water used in the city of Kathmandu is derived from groundwater.

Studies indicate that ground water pollution comes from numerous sources as we dump more of our wastes into 1) shortage lagoons, 2) septic tanks, 3) landfills that after 20-40 years can corrode and leak. Ground water in aquifers is easy to deplete and pollute because much of it is renewed very slowly. For example, the average recycling time for ground water is about 1400 years, compared to only 20 days for river water (Miller, 2002).

Surface water: Surface water originates from rain water. It is the main source of water supply in many areas. Examples of surface water include rivers, banks, man-made reservoirs and sea water. Surface water is prone to contamination from human and animal sources. As such it is never safe for human consumption unless subjected to sanitary protection and purification before use. In general, surface water supplies posses a high probability of organic, bacterial and viral contamination (Park, 2005).

) Traditional Water Supply System in Lalitpur Sub-Metropolitan City

The traditional water supply system from stone spouts and dug wells are the significant alternatives for people of Lalitpur Sub-Metropolitan city. The dug wells are a key water resource largely over looked until now (Joshi, 1993).

Stone spouts: Stone spouts are the flow ground water sources. These are constructed of the horizontal brick channel. These are used before the city water supply system was established. Use of these stone spouts is still in some areas for drinking and other domestic purposes due to the scarcity of city water supply. The spouts are located within rectilinear pits built into the ground and supplied through 'Raj Kulo' (state canals), which met irrigation needs using local water sources. Even though they received state sanctions, they were decentralized in operation (ICIMOD/MOEST/UNEP, 2007).

Well: In this urban setting still number of open and closed wells could be seen. Although most of the previously opened wells are closed and fitted with tube wells, some of them are still using the water directly from open wells. Most of the open wells are 10-20m deep. Community people believe that the wells should be regularly used because if wells are used regularly, water becomes clear and clean. This type of well water can be used for drinking also. But most of the community well as well as personal well water is being used for other purposes besides drinking such as washing utensils, clothes, taking bath and to use in the toilet.

Water Quality

Water quality is the physical, chemical and biological characteristics of water in relationship to a set of standards. Parameters for drinking water quality typically fall under two categories: chemical/physical and microbiological. Chemical/physical parameters include heavy metals, trace organic compounds, total suspended solids (TSS), and turbidity. Microbiological parameters include Coliform bacteria, *E. coli*, and specific pathogenic species of bacteria (such as cholera-causing *Vibrio cholerae*), viruses, and protozoan parasites.

Originally, fecal contamination was determined with the presence of coliform bacteria, a convenient marker for a class of harmful fecal pathogens. The presence of fecal coliforms (like *E. Coli*) serves as an indication of contamination by sewage. Additional contaminants include protozoan oocysts such as *Cryptosporidium* spp, *Giardia lamblia*, *Legionella*, and viruses (enteric). Microbial pathogenic parameters are typically of greatest concern because of their immediate health risk.

Water quality has deteriorated due to rapid industrialization, population growth, and intensive agriculture as they generate increasing quantities of industrial waste water, domestic waste and agriculture run-off. Water get contaminated either at source or while passing through water pipes which are poorly laid and maintained, or in the homes when it is not stored properly (Raghu Ram, 2005).

Bacteria and Other Micro-Organisms in Drinking Water

A biological agent is defined as a microorganism that either causes disease in man, plants, and animals or causes the deterioration of material. Drinking water can contain a number of different organisms, including bacteria, viruses and parasites, which can cause disease. These organisms can exist in the source water and survive through treatment, or they can enter the water supply in the distribution system. Well water can be contaminated if the well is not built properly or if it draws on water on the surface of the ground, such as shallow wells or wells drilled in (fractured) rock. Surface water, such as rivers, lakes and streams, can also contain disease causing organisms from various sources.

Sources of Microbes in Drinking Water

Human and animal wastes are a primary source of microbes in water. These sources of microbial contamination include runoff from feedlots, pastures, dog runs, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities, and natural soil/plant bacteria. Microbes from these sources can enter wells that are either open at the land surface, or do not have water-tight casings or caps.

Insects, rodents or animals entering the well are other sources of contamination. Old wells were dug by hand and lined (cased) with rocks or bricks. These wells usually have large openings and casings that often are not well-sealed. This makes it easy for insects, rodents, or animals to enter the well.

Another way microbes can enter a water supply is through inundation or infiltration by flood waters or by surface runoff. Flood waters commonly contain high levels of microbes. Small depressions filled with flood water provide an excellent breeding ground for bacteria. Whenever a well is inundated by flood waters or surface runoff, bacterial contamination is likely. Shallow wells and wells that do not have water-tight casings can be contaminated by microbes infiltrating with the water through the soil near the well, especially in coarse-textured soils.

Older water systems, especially, dug wells, spring-fed systems and cistern-type systems are most vulnerable to bacterial contamination. Any systems with casings or caps that are not water-tight are vulnerable. This is particularly true if the well is located so surface runoff might be able to enter the well. During the last five to 10 years, well and water distribution system .construction has improved to the point where biological contamination is rare in newer wells.

) Situation of Drinking Water Supply System in Nepal and Lalitpur Sub Metropolitan City

Nepal is rich in water resources but planned water supply was started only in 70's. Access to drinking water is measured by the number of people who have a reasonable means of getting an adequate amount of water that is safe for drinking, washing, and essential household activities. As a country's economy becomes stronger (as its GNP per capita or PPP rise) a larger percentage of its people tend to have access to drinking water and sanitation. The national coverage of water supply system was only about 4% in 1970 and now increased to 70% (ENPHO, 2005c). However, there are still several hilly regions where people have to spend hours to collect drinking water. Rural communities continue to use most convenient sources of water irrespective of quality. Sanitation facility is still in very poor condition having only 20.9% national coverage (Water Aid, 2005).

The main reason for poor access to safe water is the inability to finance and to adequately maintain the necessary infrastructure. Overpopulation and scarcity of water resources are contributing factors.

Table 1. Drinking Water Situation in Nepal.

Source	% of People Served
Piped water	31.5
Well water	7.1
Hand pump	31.9
Spring water (Kuwa)	18.9
River/stream	7.2
Others	3.4
Population with Access to Safe water	42 %

Source: MoH, (2004)

Lalitpur sub-metropolitan city has a long history of planned and well-designed water supply system as part of massive urban infrastructure of those times fulfilling the water need of its people dating back to Lichhavi period. And more remarkable is the fact that many of these systems are still functioning and contribute significantly to the day to day water demand of city.

However, the present water supply condition in LSMC and the whole of Kathmandu valley as such is unsatisfactory with acute shortage in quantity, and also the quality is far from satisfactory. As Nepal's population grows, the municipal systems can not keep up the demand, so there is a continuing and even increased reliance on private wells and public dharas (stones pouts tapping ground water). The rapid and unplanned urbanization of Kathmandu valley in the past few decades have taken its toll on urban infrastructures and more so in its water supply capacity, which is strained to its limit at present and LSMC is no exception.

) Waterborne Diseases

Waterborne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated drinking water is consumed. Contaminated drinking water, used in the preparation of food, can be the source of foodborne disease through consumption of the same microorganisms. According to the World Health Organization, diarrhoeal disease accounts for an estimated 4.1% of the total DALY global burden of disease and is responsible for the deaths of 1.8 million people every year. It was estimated that 88% of that burden is attributable to unsafe water supply, sanitation and hygiene, and is mostly concentrated in children in developing countries. Waterborne diseases are among the leading causes of morbidity and mortality in low- and middle-income countries, frequently called developing countries.

This is one of the major reasons for high infant mortality rate, 74/100 live births. Similarly, diarrhea diseases alone kill 44,000 children and thousands of people suffer from water borne epidemic annually (DoHS, 2005).

Over 50,000 children die each year in Nepal from lack of access to safe drinking water. The much higher incidence of non-fatal gastrointestinal disease caused by unsafe drinking water results in high social and economic costs in lost productivity, educational deficits, and reduced quality of life.

Waterborne disease can be caused by protozoa, viruses, bacteria, and intestinal parasites.

) Prevalence of Water Borne Diseases in Nepal

Outbreak of waterborne epidemic is rampant in Nepal as in most of the third world countries. Mortality and morbidity due to such disease still top the list. Every year the onset of the epidemics comes also with the monsoon (Sharma, 2002). Many outbreaks of water borne diseases probably are not recognized; therefore, their incidences are not reported.

But there are real incidents of water borne diseases, in which improvements in drinking water quality could have saved many lives. As mentioned in the UNICEF situation analysis (UNICEF, 1987), in Nepal water and hygiene related diseases are responsible for 15% of all cases and 8% of all deaths in the general population. In 1985, over 50% of hospital patients in Nepal were found to be suffering from gastrointestinal disorder normally caused by water borne pathogens. In 1990, cholera outbreaks during summer hit different parts of the country including capital city and caused an enormous loss of lives (DISVI, 1990). In Pokhara, more than 50% of the leading diseases causing morbidity were recorded to be waterborne. In 1990, Public health division recorded 23,888 gastro-enteritis cases in 39 districts with maximum 8,437 in the Kathmandu valley (DoHS, 1998). In 1999 as well, the disease started to spring up at the beginning of the summer, striking badly the western, mid-western, and central region of the country, which the eastern region was less affected. In between April to august, public health division reported 43,520 gastro-enteritis cases with 1252 deaths (ENPHO/DISVI, 1991).

The outbreak of gastro-enteritis in eleven districts of the kingdom was recorded 2300 cases with 69 deaths in between March-April, 1998 (Gorakhapatra, 1998). Kathmandu valley is affected by the severe water crisis. The shortage of water is a problem in itself, but besides the quantitative shortage, the risk of water borne diseases has become an even serous problem. People are not having access to hygienic drinking water.

Outbreak of diseases like typhoid fever, cholera, dysentery, worm infestation, hepatitis and many other are spread by contaminated water, and are prevalent in urban and rural areas. A report from ministry of health 0.27% of patients had typhoid, 1.63% from diarrhea, and 0.07% from Jaundice and Hepatitis in Kathmandu in 2002/03, In Lalitpur, typhoid cases were 0.58%, diarrhea 2.60% and Jaundice and infective hepatitis 0.09% (MoH, 2004).

⟩ Significance of the Study

Nepal's population has grown by 28% in the past decade and about 50% of the population of Nepal relies on ground water for drinking water supply.

As Nepal's population grows, the municipal system cannot keep up with the demand, so there is a continuing and even increased reliance on private wells and public dharas (stone spouts tapping ground water). Many people face severe health threats stemming from water contaminated by sewage, agricultural practices, and industry. Over 26% of the total ground water withdrawn from Kathmandu valley is from private wells, and overall half of these are very shallow and prone to contamination. Only because of the consumption of contaminated water a large number of lives have been lost every year from Nepal.

There is an overall lack of water-quality data for Nepal and hence assessment of the main quality problem is difficult. Moreover, the water quality data of different sources used in Lalitpur is very limited. People are totally unaware about the biological contamination of water and its importance to their health.

People are using water from various sources without any treatment. They store water in any type of container even without proper cleaning. Hence there is an urgent need to determine the status of water used in Lalitpur Sub-Metropolitan city. Therefore the study was conducted in different locations of LSMC, collecting water from various sources which are using by the community people.

⟩ Limitations of the Study

The limitations of the present study are mentioned as follows.

- This study was limited to ward no.5, 13, 21 and 22 only. Thus the findings may not generalize the whole Lalitpur Sub-Metropolitan city.
- There was the limitation of time in the present study.
- The study was only focused on Bacterial contamination of drinking water and the presence of Helminths

2. OBJECTIVES

⁾ General Objective

To examine the microbiological quality of drinking water supplied in Lalitpur sub-metropolitan city.

⁾ Specific Objectives

- To find out bacterial contamination in drinking water.
- To study the presence of protozoa and helminthes in drinking water.
- To determine the seasonal variation of biological contamination of various water sources of Lalitpur sub-metropolitan city.

3. LITERATURE REVIEW

) In Global Context

Laluraj *et al*. (2006) studied the ground water chemistry of shallow aquifers which lie along the coastal zone of central Kerala. Results in general indicated that the ground waters in shallow aquifers were found to be deteriorated. The presence of *E. coli* in all dug wells indicated potentially dangerous faecal contaminations, which require immediate attention.

Karanis *et al*. (2006) estimated the occurrence of *Giardia* and *Cryptosporidium* from Russia and Bulgaria. A total of 166 samples of different origins (surface, tap, bottled, well, spring and waste water) were analyzed. Out of which 16 were positive for *Giardia* and 30 positive for *Cryptosporidium*. This study sowed that drinking water supplies in the countries are subjected to contamination with these micro-organisms with potential hazards for public health.

Sinha and Saxena (2006) calculated water quality index of underground drinking water at 10 different sites in Hasanpur, India in the pre monsoon season as well as after the onset of monsoon. Result indicated that almost all the sites were found to be highly contaminated except a few sites, where it was found moderately contaminated for both the periods during the year 2005.

Arvanitidou, *et al*. (2005) studied on the diversity of *Salmonella* spp. In northern green rivers and their correlation to faecal pollution indicators. The prevalence and diversity of *Salmonella* spp and their correlation with fecal pollution indicators (total Coliforms, fecal Coliforms, *Enterococci*) and total heterotrophic bacteria counts were investigated in 95 water samples from the northern Greek rivers Aliakmon and Axios. *Salmonella* spp were isolated in 27.4% of the samples and a total of 19 serotypes were identified. The frequency of *Salmonella* isolation was higher in the Axios (36.8%) than in the Aliakmon (21.0%) river. Significantly ($P<0.001$) more *Salmonella* spp were recorded during warm (41.4%) than cold (5.4%) months. *Salmonella* positive samples showed significantly higher counts of total heterotrophic bacteria and coliforms. The result of this study indicate that these rivers may be pathways for human and other animal contamination with *Salmonella* spp contribute to the pollution of marine waters and the surrounding environment.

Craun *et al*. (2005) reviewed the causes of outbreaks associated with recreational water during 1971-2000. A bacteria or protozoan etiology was identified in three quarters of the outbreaks. The most frequently identified agents were *Cryptosporidium* (15%), *Pseudomonas* (14%), *Shigella* (13%), *Naegleria* (11%), *Giardia* (6%), and toxigenic *E. coli* (6%). Outbreaks attributed to *Shigella*, *E. coli* 0157:H7 and *Naegleria* were primarily associated with swimming in fresh water such as lakes, ponds and rivers.

In contrast to outbreaks caused by *Cryptosporidium* and *Giardia* were primarily associated with treated water in swimming and wading pools. Important sources of contamination for both treated and untreated recreational water were the bathers themselves. Contamination from sewage discharge and wild or domestic animals were also important sources for untreated waters.

Godfrey *et al*. (2005) studied the relationship between rainfall and microbiological contamination of shallow ground water in Northern Mozambique. 25 wells were monitored over a twelve month period and than compared to historical rainfall from the previous 8 years. The study drew three distinct conclusions. Firstly the study demonstrated the direct pulse response between increased numbers of presumptive thermo-tolerant coliforms and enterococci bacteria. Secondly, the study observed high risk of contamination through localized, as opposed to aquifers pathways, and thirdly, the study noted higher survival function and stability of enterococci bacteria as compared to thermo-tolerant coliforms in the environment and at depth.

Schraft and Watterworth (2005) studied enumeration of heterotrophs, fecal coliforms and *E. coli* in water. A total of 177 naturally contaminated water samples were analyzed by membrane filtration. Heterotrophic counts were between 10^3 and 10^4CFU/ml and average $\log_{10}$ counts obtained petrifilm (TM) EC plates were slightly lower that on MFC agar with a correlation coefficient of 0.949. The average $\log_{10}$ counts for conformed *E.* coli petrifilm (TM) EC coefficient of 0.879. Specificity of petrifilm (TM) EC plates and MFC agar was evaluated by comparing typical colony counts with confirmed counts. On MFC agar, counts per typical colonies were by 2 $\log_{10}$ CFU higher than the actual conform counts. In contrast on petrifilm(TM) EC plate's typical colony counts were almost identical to confirmed colony counts for both fecal coliform and *E. coli*. These comparisons illustrate the high specificity of petrifilm (TM) EC plates for enumeration of both fecal coliforms and *E. coli* in water.

Senhorst and Zwolsman (2005) studied the water quality during periods of low flow and extreme heat, which are assumed to increase in frequency and intensity due to climate change. The results indicated that the impact of climate change on water quality can not be generalized and should be assessed on a case by case basis. However the impact of extreme situations (floods and draughts) seems to be largest whilst water quality under average discharge conditions appears to be relatively unchanged.

Tallon *et al*. (2005) studied on fecal contamination of drinking water has caused numerous disease outbreaks. Because the risk of disease outbreaks correlate with the incidence of fecal contamination, fecal bacteria, are used as indicators of fecal contamination and hence the possible presence of disease causing organisms. However, different microbiological fecal indicators are used in different countries and jurisdictions. Therefore it is important to understand the potentials and limitations of these indicator organisms. Before realistically implementing guidelines and regulations to safeguard our water resources. This review considers the history of indicator organisms, the evolution of the analytical methodologies and addresses the advantages and limitations of current fecal indicator micro organisms.

Giatti *et al*. (2004) studied the basic sanitary conditions in Iporanga, Sao-Paulo, Brazil to assess the pollution of water bodies by domestic sewage and to evaluate the basic sanitary conditions of residences and knowledge of the local population concerning intestinal parasitic disease and the hazards they present to public health. 13 water samples were analyzed from four sites. Rates of total and faecal coliforms were measured and median value was presented so as to show domestic sewage contamination in the area. 50% of the local house holds were surveyed. Results showed microbiological indexes indicative of pollution by domestic sewage. In 91% of the households investigated, sewage disposal was found to be the major contributing factor in the contamination of the environment. Results concluded that the risk of intestinal parasites and water borne diseases is aggravated by increase in population.

Horman *et al*. (2004) studied a total of 132 surface water samples form 7 lakes and 15 rivers in south western Finland during 5 consecutive seasons form Autumn 2001 for the presence of various entero pathogens and fecal indicators. All together 41% of the samples were positive for at least one of the pathogens; 17.3% were positive for Campylobacter spp, 13.7% were positive for *Giardia* spp, 10.1% were positive for *Cryptosporidium* spp, and 9.4% were positive for nonoviruses. The samples were significantly positive for enteropathogens ($P<0.05$) less frequently during the winter season than during the other sampling seasons.

Kara *et al*. (2004) studied the bacteriological quality of municipally supplied and of well water of the towns and city center in the province of Nigde and Turkey. 70 samples were collected. In the study 23 water samples were found potentially unsafe because of the existence of coliform bacteria in them, which was probably caused by the inexistence of sufficient chlorination.

Paul *et al*. (2004) studied microbiological condition of urban ground water in the vicinity of Leaky sewer system. The possible deterioration of the microbiological quality of ground water due to leaky systems was investigated in a medium sized city (Rastatt) in SW-Germany. The sampling was performed in March and in July and in October. Coliform, *E. coli* and Enteroccocci were enumerated as indicator organism for waste water contamination. In addition, the total number of colony forming units was determined. Bacterial counts of coliform and *E. coli* generally increased during the course of the investigation (March to October). The contamination with fecal indicator bacteria was greater adjacent to leakage location. In addition, elevated concentration of Ammonium ions were observed at these areas. The result provided evidence that leaky sewer system elevate fecal indicator concentration in ground water with associated with potential health risks were such water are used for potable water supply.

do Amaral *et al*. (2003) studied the drinking water in rural farms of Sao-Paulo. A total of 180 drinking water samples from sources, reservoirs, and water from site of consumption were collected in 30 farms. The results showed that 90% of drinking water samples from sources, 90% from reservoirs, and 96.7% from sites of consumption, collected during the rainy season, and 83.3%, 96.7%, and 90% of samples collected in dry season were below the quality control standards for drinking water.

Momba *et al*. (2003) investigated the effect of long storage and house hold containers on the microbiological quality of water in a rural community of South Africa. Borehole water from the reservoir and seven stand pipes were used. The results revealed three factors that affect the microbiological quality of drinking ground water used by the rural community: the intake water, the duration of storage, and household containers. The initial levels of faecal coliform and HPC bacteria, in both reservoir and stand pipe water far exceeded the limit. The persistence of faecal coliform in polyethylene- stored water was observed in 72 hours of storage whereas in galvanized steel stored water it was completely eliminated after 48 hours. *E. coli* was the most dominant faecal coliform found in the initial and stored waters. Higher number of HPC bacteria in the water stored in polythene was observed than in the water stored in galvanized steel. HPC bacteria were also significantly different in household container- stored water from standpipes.

Al-Thani (2003) conducted the microbiological analysis of the waste water and its sediment around Abu-Harmour pond and Abu-Nakhal pond, located oil and outskirts of Doha city, and revealed that Coliform bacteria were prominently present in the former than in the later one. *Escherichia coli* was present in the coastal waste water in all sites around both ponds. Yet, *E. coli* was present in wet soils around these ponds primarily near the discharge sites where new water is constantly poured in. Also the following Abu-Nakhal pond: *E. coli*, *Salmonella* spp, *Oritus* spp, *Cromobacterium violaceum*, *Klebsiella pneumonis*, *Aeromonas* spp, *hydrophilia* spp, *Pseudomonas aeruginosa*. Moreover *Streptomyces, Bacillus*, and *Macrococcus* spp were more prominent in the sediment around Abu-Harmour pond than Abu-Nakhal pond.

Abdel (2001) conducted bacteriological and chemical evaluation of drinking water in Qalaubiya, Egypt. 32 water samples were taken, 28 from hand pumps used for human consumption and 4 from ordinary tap water. These were then subjected to bacteriological and chemical contamination. The investigated water samples were evaluated for their validity for human consumption. The result revealed that most water samples contained high number of total coliform bacteria which exceed the permissible limit recommended by WHO (1988). Faecal coliform was recorded in majority of water samples while faecal stereptococci were detected in a few numbers of water samples. Majority of tap water samples were found bacteriological and chemically safe, recommendation were suggested for the treatment of hand pump water produced at depths of (10-23m) in a trial to prevent human and animal illness.

Grover and Thakur (2001) conducted bacteriological analysis in Shimla drinking water. They took 300 water samples were collected form piped supply and 20 different natural sources. They were analyzed bacteriological for four important bacterial indicators every month over a period of one year. The MPN of total coliform, *E. coli*, *Streptococcus faecalis* were detected by multiple tube method and *Clostridium perfringens* was isolated using litmus milk medium. From piped supply only one sample was unfit for human consumption. About $1/3^{rd}$ of samples showed presence of *Clostroidium perfringens*. *Salmonella typhii* was isolated in 1.25% samples by membrane filtration technique. The water from all the natural sources was unfit for human consumption whereas piped water supply was of good quality in general.

Roberts *et al*. (2001) conducted study to assess the ability of a water container with a cover and a spout to prevent household contamination of water in a Malawian refugee camp. Contamination of water in homes was found to be the main cause of outbreak of cholera and diarrhea water. Flowing from the source wells had little or no microbial contamination; water collectors contaminated their water, primarily through contact with their hands.

Clang (2000) in her article "Sydney's 1998 water quality crisis" described about a drinking water quality crisis, which occurred in Sydney, Australia, from July through September 1998. High concentrations of *Cryptosporidium* oocysts and *Giardia* cysts were repeatedly observed in water samples collected in the distribution system. Although no increase in water borne diseases were detected. The reported concentrations of both cysts and oocysts ranged from non detect to thousands of parasites per 100L of finished water.

Francy *et al*. (2000) studied the occurrence and distribution of microbiological indicators in ground water (143) and stream water (136) samples. They found total coliforms in 99%. *Escherichia coli* in 97% and *Clostridium perfringens* in 73 of stream water samples. Total coliforms were found in 20%, *E. coli* in <1% and *C. perfringens* in none of the ground water samples.

Haas (2000) studied the epidemiology, microbiology, and risk assessment of water borne pathogens including *Cryptosporidium*.

Jarmey *et al*. (2000) described about a novel method for detection of viable *Giardia* cysts in water samples. Assessment of *Giardia* viability is major requirement for public health surveyors and the water industry. Survey indicators of viability such as stains, excystation and animal insecurity have been used to enumerate cysts with varying degree of success. A combined detection- viability method for use in water samples would be useful for detecting and determining the viability of cysts in raw and drinking water and the efficacy of disinfection at treatment plants.

Kravitz *et al*. (1999) conducted quantitative bacterial examination of domestic water supplies in the Lesotho highlands: water quality, sanitation and village health. Their report is as follows: a total of 72 villages water sources were classified as unimproved (n = 23) and semi-improved (n = 37), or improved (n = 12). Based on the estimation of total coliforms, which is a non-specific bacterial indicator of water quality all unimproved and semi-improved water sources would be considered as not potable. *E. coli*, a more precise indicator of faecal pollution was absent ($P<0.001$) in most of the improved water sources. Among 588 queried households, only 38% had access to an improved water supply. Sanitation was a serious problem, e.g. fewer than 5% villagers used latrines, and 18% under 5 years old had suffered a recent diarrhoeal illness.

Musa *et al*. (1999) studied water quality at the source and point of consumption among rural and peri-urban communities in Northern Sudan. The result showed both water sources and water stored for consumption had faecal coliform counts in excess of WHO standards, with higher counts at the end of the rainy season.

Bilgrami and Kumar (1998) studied about the bacterial contamination in water of the river Ganga and its risk to human health. The result showed that water of the river Ganga is extensively used for drinking, bathing and cleaning purposes. Bacterial analysis of Ganga water was carried out at three different sites in Bhagalpur town which discharges interrupted municipal and industrial effluents in river at various points. Total bacterial density (TBD), total coliform (TC), faecal coliform (FC), faecal streptococci (FS), *E. coli* and *Clostridium perfringens* were substantially high and much beyond the permissible limit of WHO. There was a marked correlation between physic-chemical quality water and bacterial density. The presence of *Actinomyces* spp, *Aerobacter aerogenes*, *A. cloacoe*, *Micrococcus* spp and *Shigella* spp indicated the level of faecal contamination in water. Hence, direct consumption of untreated Ganga water and bathing in this stretch poses a great health risk.

Gibson (1998) worked on combined sewer outflows: a source of *Cryptosporidium* and *Giardia*. They reported that the *Cryptosporidium* oocyst and *Giardia* cyst were commonly observed in the urban stream during dry weather conditions, with concentrations of 5-105 oocyst/ 100L and 13-6579 cysts/ 100L respectively. Preliminary study suggests that CSOs may significantly contribute to the load of *Cryptosporidium* and *Giardia* in ambient water and resource water utilized for recreational and potable water. However, further investigation will be needed to determine and characterize the full effect of these apparent loading sources.

Mugga (1998) reported the most country areas of Uganda suffer form a serious lack of adequate water supply. A lack proportion of the population dose not have reasonable access to lack water and is without facilities for hygiene water disposal, this results in the spread of various water borne diseases problems encountered by the people are shortage of water during dry seasons, long daily track to carry water from the sources and pollution of surface water which encourages diseases such as schistosomiasis and dysentery.

Borrego and Figueras (1997) carried out an experiment on microbiological quality of natural water several aspects of the microbiological quality of natural water especially recreational water has been reviewed. The importance of the water as a vehicle and/or a reservoir of addition, the concepts, types, and techniques of microbial indicators and index microorganisms are established. The most important differences between fecal streptococci and enterococci have been discussed, defining the concept and species included. In addition, we have revised the main alternative indicators used to measure the water quality.

⟩ In Nepalese Context

Warner *et al*. (2008) studied water quality in Nepal's Kathmandu Valley from more than 100 sources including municipal taps, dug wells, shallow-aquifer tube wells, deep-aquifer tube wells, and stone spouts. Most problematic were total coliform and *Escherichia coli* bacteria, which were present in 94 and 72% of all the water samples, respectively.

Significant differences existed in contamination levels between types of sources; dug wells and dhunge dharas, being the shallowest, were the most contaminated by bacteria and nitrate; deep-aquifer tube wells were the most contaminated by arsenic. Whereas *E. coli* concentrations decreased with depth, iron and ammonia concentrations increased with depth.

Bajracharya (2007) conducted drinking water quality of Kathmandu Metropolitan city. For which, 114 samples were analyzed for bacteriological to assess the drinking water quality. The bacteriological analysis of water samples revealed the presence of total coliform in 90.35% of total samples (tube well 97.37%, tap-73.68% and stone spout 100%) revealing the fact that the drinking water quality is not microbiologically safe.

Joshi *et al*. (2007) tested 138 water samples from Kathmandu Metropolitan City – 19 and 20 for the assessment of water borne protozoan and helminth parasites. They observed cysts of *Giardia* spp (4.35%), cysts of *Entoamoeba histolytica* (5.07%), oocysts of *Cryptosporidium* spp (54.35%), eggs of *Ascaris* spp (34.78%), eggs of *Taenia* spp (5.07%), eggs of *Gnathostoma* spp (5.07%), eggs of *Enterobius* spp (6.52%), eggs of *Strengyloides* spp (0.72%), eggs of *Trichuris* spp (13.04%), eggs of *Toxoplasma* spp (6.52%), eggs of *Toxocara* spp (5.8%), and eggs of *Ancylostoma* spp (2.17%).

Prasai *et al*. (2007) conducted a study to evaluate the quality of drinking water of the valley. A total of 132 drinking water samples were randomly collected from 49 tube wells, 57 wells, 17 taps and 9 stone spouts in different places of Kathmandu valley. The samples were analyzed for microbiological parameters. Total plate and coliform count revealed that 82.6% and 92.4% of drinking water samples found to cross the WHO guideline value for drinking water. During the study, 238 isolates of enteric bacteria were identified, of which 26.4% were *Escherichia coli*, 25.6% were *Enterobacter* spp, 23% were *Citrobacter* spp, 6.3% were *Pseudomonas aeruginosa*, 5.4% were *Klebsiella* spp, 4.0% were *Shigella* spp, 3.0% were *Salmonella typhi*, 3.0% were *Proteus vulgaris*, 3.0% were *Serratia* spp and 1.0% were *Vibrio cholerae* .

NGO Forum (2006) concluded study on traditional stone spouts of five municipal area of Kathmandu valley. The study was conducted in dry season (March to May) and wet season (July to September) for 84 samples from different places of Kathmandu valley. During the coliform test for 72 hours observation, only one sample showed negative result while all the 83 samples have positive test result i.e. coliform is present.

ENPHO (2005a) conducted ground water quality surveillance in Kathmandu and Lalitpur Municipal areas in October 2004 to March 2005. A total of 351 and 331 water samples were collected from deep tube wells (NWSC and private), shallow tube wells and shallow dug wells in the wet and dry seasons respectively. A high percentage of the samples tested were faecally contaminated. Despite the increase depth of wells, deep tube well water (33.9%) was contaminated with *E. coli*. High percentage of shallow dug wells (44%) was faecally contaminated compared to shallow tube wells (9.4%)

ENPHO (2005b) collected a total of 78 water samples from NWSC supply network from different areas of five municipalities in order to regular monitor the faecal coliform count in the supplied water in order to aware the general public on the quality of water being supplied and to draw attention of the concerned authority. It revealed the fact that in Kathmandu (57.8%) Kirtipur (80%) Lalitpur (6.7%), Bhaktapur (20%) and Thimi (0%) of total water samples were faecally contaminated.

ENPHO (2005c) analyzed water samples from different sources, reservoirs/tanks and taps for physico-chemical and bacteriological parameters. Bacteriologically, high proportion of water samples (sources, reservoirs, and taps) was found to be unacceptable. Out of 32 sources, 93.6% water samples were found to be contaminated with total coliforms, and *E. coli* was present in 78.1% samples. Similarly, out of 15 reservoirs, 86.7% samples were found to be contaminated with total coliforms and *E. coli* was present in 66.7% samples. Out of 128 tap samples, 82.8% samples were found to be contaminated with total coliforms, while *E. coli* was present in 56.3% samples. Bacterial counts were high in some samples tested from Banepa, Bhairahawa, Mahendra Nagar, Nepalgunj, Pokhara, Panauti and Taulihawa. Bacterial counts were high for some samples compared to other taps in some municipal areas despite the same water distribution line, suggesting the contamination through leakage or seepage in the supply system or intrusion of waster from external environment.

Pradhan *et al*. (2005) conducted an investigation on the quality of drinking water used by the communities of Bungmati, a rural town of Kathmandu Valley, Nepal and of local knowledge of water quality and water borne diseases. The results showed that the values of the bacteriological parameters such as coliform bacteria and *Escherichia coli* are such that the drinking water is not potable in terms of bacteriological point of view.

Rainey and Harding (2005) examined pH, Turbidity, and faecal contamination of drinking water form household water storage containers, wells and taps, and the Godawari river, and tested the effectiveness of solar disinfection (SODIS) in reducing level of faecal contamination from household containers. The result showed that untreated drinking water was found to have levels of contamination ranging from 0-100 numerous to count faecal coliform CFU per 100ml. Source water was significantly more contaminated than water from the household storage containers, wells were less contaminated than taps.

Joshi and Baral (2004) carried out the study regarding chemical and microbial quality of ground water of Kathmandu valley. They analyzed 160 samples randomly collected from 86 tube wells and 77 open wells in urban areas and reported that more than 87% of analyzed ground water samples of tube wells and open wells were found to be contaminated with faecal pollution indicator organisms "Coliform bacteria" and hence unfit for drinking.

Joshi and Maharjan (2003) carried out an inventory and tested water samples in ward no 19 and 20 of Kathmandu Metropolitan city. Over 150 different water samples including traditional community taps, household connections, shallow wells, deep wells, and household water storage tanks, were analyzed using a low cost bacterial test (H_2S) prepared locally. High bacterial contamination was detected during spring and monsoon periods in most water sources (over 90% of stone taps and wells). The stone tap water is less contaminated than tube well water. While there is no significant difference between NWSC supplied direct tap water and NWSC supplied stored water. The water in the distribution system was slightly better with 70% of household taps in ward 19 and 30% of taps in ward 20 exceeding national drinking water and WHO standards i.e. *E. coli* count is <3/100ml of water.

Schaffner (2003) studied different water sources including springs, taps, dug wells and water harvesting jars in selected areas of the Jhikhum Khola watershed. She concluded that microbial contamination is the dominant parameter of concern in all drinking water systems, with the highest contamination risk during the pre-monsoon and monsoon seasons.

Thapa (2002) studied on the biological contamination in drinking water sources of Kathmandu. Total of 75 water samples were tested for bacteriological pollution, these includes ground water sources such as tube wells and wells and surface water sources such as stone taps. 83% of the water sources tested was found to be bacteriologically polluted and not safe for drinking.

Maharjan *et al*. (2001) tested water samples in 6 VDC's of western part of Kathmandu and in ward 20 KMC. 60% of the samples tested were found to be highly polluted with bacteria at a level of greater than 100 bacteria per 100ml of water. 32% of the samples had more than 10 bacterial per 100 ml and 4% had less than 10 bacteria per 100ml. In urban area of ward 20 KMC, among the water samples taken, 87.5% were found to be moderately polluted at a level of less than 100 bacteria per 100 ml and none of them were found to be free of pollution.

Regmi (2001) analyzed water quality of stone spouts of Kathmandu. The microbiological pollution was observed more in monsoon season. Stone spouts are microbiologically unsafe.

Warner *et al*. (2001) invested Groundwater quality in Kathmandu and reported every drinking-water source had total coliform contamination, but significant differences in contamination levels between public stone spouts, municipal tap water, dug wells were observed. The private tube wells; dug wells are the most contaminated. Statistical analyses show increasing Fe content with depth and an inverse relationship between Fe content and fecal coliform levels.

ENPHO (2000) from 1988-1996 periodically analyzed drinking water quality of Kathmandu supplied by NWSC. The report indicated that pipe water supply system in Kathmandu city is microbiologically unsafe for drinking. Mostly, water was found to be faecally contaminated.

ENPHO (2001) carried out special test of 24 stone spouts around Kathmandu city. The result showed that water is bacteriologically contaminated, faecal coliform ranging from 2-549 Coliform/100 ml more than the WHO guideline value.

Maharjan and Sharma (2000) conducted study on bacteriological quality of ground water in urban Patan and antibiotic sensitivity against isolated enteric bacteria. A total of 70 water samples were randomly collected and analyzed for bacteriological quality. 85.6% of the samples showed presence of total Coliform and 68.6% contained faecal coliform. In this study 120 enteric bacteria were isolated from 49 samples.

Bottino *et al*. (1999) carried out the study regarding the water quality of stone spouts in Kathmandu city and reported that bacteriologically all the samples shown faecal contamination in both seasons, hence water from all the stone taps were unfit for drinking.

Global Resources Institute and the International Buddhist Society, during April 1999, sampled and tested water from nine villages in the Lumbini area. Single water samples were taken from representative water sources in eight of the 17 villages served and in Lumbini. The water samples were contaminated from a total of 42 sources of drinking water including tube wells, one artesian well, open wells, and river water. I all cases the water was being used for drinking water with out boiling or other forms of disinfection. The water obtained from this well consistently tested with *E. coli*. Count in the 20 to 30 colony forming units per 100 ml. range.

NESS (1999) conducted water quality of surface water, well water and tap water of Madhyapur Thimi in two phases from October 1998 to February 1999. 5 samples were taken from well and 2 samples were from tap. In first phase, the bacteriological parameter testing total coliform count, all the water samples bacteriologically unsafe, crossing WHO guide live value.

Maharjan (1998) examined 70 water samples randomly collected from 5 shallow pumps, 3 shallow wells, 14 stone spouts and 48 dug wells in urban area of Patan city. Out of these, 85.6% samples showed presence of total Coliform and 68.6% contained faecal Coliform.

Ghimire (1996) studied the water quality of 6 stone spouts and 5 dug wells at the Patan area during two seasons. The study reported 2 stone spouts uncontaminated, 1 spout showed 1000 col/100ml, 2 spouts with more than 1,000 col/100ml and 1 spout was dry during summer. One dug well was free of contamination ad rest 4 wells were highly contaminated (100-7,000 col/100ml). All the spouts and wells were heavily contaminated during rainy season (4-2,600 col/100ml).

Pradhan *et al*. (1995) carried out a study on water quality and people's knowledge about water related diseases in Kirtipur locality. The study indicated that the quality of water in most places of Kathmandu is not acceptable. Bacteriological analysis of water tested on parameters such as total heterotrophs, faecal coliform and *E. coli* indicated that the public water supply was far from being satisfactory.

Khadka (1994) studied the quality of water supply in Kathmandu valley and reported the Quality of raw water from surface water sources varies seasonally. Generally it is more turbid and contaminated with bacteria in rainy season. Groundwater from limestone aquifers in the southern part of the valley is good in quality but waters from other well fields contain iron, manganese and ammonia in high concentration. Shallow well waters contain coliform bacteria in significant numbers.

Rawal *et al*. (1994) conducted study on bacteriological investigation of drinking water in Nepal. This study showed that out of 283 water samples only 9.9% were recognized for drinking purpose. A total of 78.8% (223/283) of water samples showed the growth of coliform bacteria. *E. coli* showed a high detection rate.

Pradhananga *et al*. (1993) examined water samples from six different stone spouts around Pashupati area on three occasions during 1992. All the stone spouts were contaminated by faecal coliform. Average values of coliform at *Ban Binayak* (44 col/100 ml), *Arun Dhara* (88 col/100ml) *Barun Dhara* (43 col/100ml), *Ganga Hiti* (10 col/100ml), *Mitra Dhara* (8 col/100ml), and *Ba Hiti* (61 col/100ml) were recorded.

Dhaubadel (1992) carried out study in 7 sectors of drinking water quality of Bhaktapur city. During the study bacterial analysis of tap water showed that bacteriological quality of tap water is far away from the safety value. The numbers of Coliform group bacteria were high in most of the sample that indicate the probability of pollution of water by sewage on the whole, in general the chemical and bacteriological contamination of water was found to be increasing more in the surface water than drinking water.

ENPHO/DSVI (1992) conducted a one year monitoring on microbiological quality of water supply in the Kathmandu city. 39 samples from 5 treatment plants and 172 samples from 37 public taps were examined from different localities. Seven samples that is, 18% from treatment plants were found contaminated with an average faecal coliform of 4 col/100ml. Similarly, 50% samples from public taps were found contaminated. The bacterial densities in contaminated samples ranged from 1 to TMTC col/100ml.

A study done by Karmacharya *et al*. (1992) in the Kathmandu showed that of 172 samples from 5 treatment plants and public taps, 50% of water samples of city water supply were found contaminated with Coliform.

Lohani (1992) conducted research on the bacteriological parameters in well water in Koteshwor area. The bacterial analysis of well water showed the quality of well water was far away from safety vale and no. of Coliform bacteria were higher.

ENPHO/DISVI (1990a) conducted a study on water quality of 21 stone spouts of the Kathmandu city. Bacteriologically, samples from all the spouts have shown faecal contamination. The faecal coliforms densities were observed in the range of 1 to 37,602 col/100ml of water. Out of total, 81% of the stone spouts showed very high contamination (>100 col/100ml) and 19% exhibited less than 100 col/100ml.

CEDA (1989) tested water samples from different locations in Kathmandu and reported that all samples were contaminated with faecal matter indicating that tap and ground water sources were unsafe for drinking.

Adhikari *et al*. (1986) carried out Coliform tests of 100 samples of drinking water (taps water natural springs and ponds) from different areas in the Kathmandu valley and found unsatisfactory results with more than 1800 coliform per 100 ml of water.

Sharma (1986) found that the level of Coliform contamination of drinking water in Kathmandu had significantly increased 4800col/100 ml of sample.

Albala and Tylor (1985) conducted a study on water quality and Diarrhoeal diseases in a rural community of Eastern Nepal. The water quality of Nundaki, a small village with separate wards in Eastern Nepal was surveyed using Millipore coliform counters. The counts from this region were low, indicating relatively clean water. Three wards were surveyed and little variation was noted. A health survey was done of the village that revealed 30 cases of diarrhea (13%). Prevalence of diarrhea ware related to type of water storage, water storage user, water usage and family size.

Leuenberger (1983) conducted a second water quality study to determine the bacteriological quality of water supplied by the CWSS programs. In total 13 schemes were covered from September to October. The study indicated the water quality was satisfactory in all 13 schemes from spring catchments to tap water and also indicated a trend of water contamination through transport and storage. Levels of faecal Coliform contamination were acceptable at the tap but rose to unacceptably high levels in storage containers. Levels of 80 faecal col/100 ml were recorded.

Bovier (1978) started water quality test in Pokhara conducted by CWSS programs as first phase of water quality to define programs success in supplying safe water for human consumption in 1978 throughout the year. 15 to 20 samples form each spot of spring catchments, storage tank, tap and in a private house were taken over a period of 9 months. The total coliform was often too numerous total count and faecal Coliform ranged between 0 and 48 per 100 ml concluding none systems met the WHO international standards.

Sharma (1978) studied the quality of drinking water supplied to the households of 39 localities of Kathmandu valley and found some degree of faecal contamination with Coliform densities ranging from 4-450/100 ml of water.

4. MATERIALS AND METHODS

Materials

For H_2S Test

Materials

1. Sample test tube with heat resistant lids
2. Clean and sterilized glass bottles (150ml to 200ml) with heat resistant lid.
3. Absorbent paper/whattman filter paper #3
4. Autoclave
5. Reagents to prepare the media in liquid form
6. Pipettes 5ml and 1ml
7. Graduated cylinder
8. 10ml Pipette graduated in 0.2ml intervals or a small graduated cylinder
9. Permanent marking pen
10. Paper foil (Aluminum)
11. Hot air oven
12. 2-3 liters of distilled, bottled or dechlorinated tap water
13. Paper towels
14. Data collecting sheets

Chemicals

1.	Bacteriological peptone	40.0g
2.	Di-potassium hydrogen phosphate	3.0g
3.	Ferric ammonium citrate	1.50g
4.	Sodium thiosulphate	2.0g
5.	Teepol	2.0ml
6.	Distilled or boiled tap water	100ml

For Sample Collection and Processing

Materials

1. Sample test tubes/bottles with heat resistant lids for about 20-30 samples.
2. Marking pencil wax and/or marking pen
3. Masking taps
4. A ruler graduated in millimeters
5. A paper towels
6. 2 or 3 liters of boiled or bottled dechlorinated water
7. Incubator
8. Data recording sheets (see appendices)

For Coliplate^{Tm} and Colistrip^{Tm} Tests

Materials

1. Coliplate^{Tm} and Colistrip^{Tm} test kits
2. Marking pencil wax and/or permanent marking pen
3. Masking tape
4. A ruler
5. Paper towels
6. One liter of boiled or bottled dechlorinated water
7. A container with contaminated water
8. Incubator
9. MPN tables for interpreting results (see appendices table 16 and 17)
10. Data recording sheets (see appendices table 21)

For Microscopic Examination

Materials

1. Microscope
2. Slides
3. Cover slips
4. Vials or bottles for sample collection
5. Test tubes
6. Pipettes and droppers
7. Centrifuge machine

Chemicals

1. Zinc Sulphate ($ZnSO_4$)
2. Saturated solution of Sodium Chloride (NaCl)

Method

Study Area and Sampling Sites

The study was conducted in Lalitpur Sub-Metropolitan City. Four different wards No 5, 13, 21, and 22 of Lalitpur sub-metropolitan city, where various sources like spouts, dug wells, hand pumps, and tube wells are used for water, were selected randomly for the study. Water samples were collected from both storage tanks of restaurants, hotels, and slaughter houses and directly from community wells, stone spouts and taps, personal tube wells. Samples were collected in two season's winter and rainy. Total 160 samples in each season were collected and analyzed as described below.

- **Brief Introduction of the Study Area**

Lalitpur sub-metropolitan city (LSMC), popularly known as Patan is currently one of the most vibrant cities of the kingdom of Nepal. It is situated in the southeast part of the magnificent Kathmandu valley which is located between the latitudes 27o 32' 13" and 27o 49' 10" North and longitudes 85o 11' 31" and 85o 31' 38" East. The valley lies at a mean elevation of about 1350 m. above sea level.

It is located in about 5 kilometers south east of Kathmandu with its urban history dating back to as far as 2300 years; LSMC is one of the three major cities located inside the Kathmandu valley, besides Kathmandu and Bhaktapur. The city spread over an area of 15.43 km^2 and is politically divided into 22 wards.

Located adjacent to the capital city of Kathmandu, LSMC has today become an integral party of the valley capital region, called greater Kathmandu, consisting of two major cities Kathmandu and Lalitpur.

LSMC has a long history of planned and well-designed water supply system as part of massive urban infrastructure of those times fulfilling the water need of its people dating back to Lichhavi period. And more remarkable is the fact that many of these systems are still functioning and contribute significantly to the day-to-day water demand of the city.

However, the present day water supply condition in LSMC and the whole of Kathmandu valley as such is unsatisfactory with acute shortage in quantity, and also the quality is far from satisfactory. The rapid and unplanned urbanization of Kathmandu Valley in the past few decades have taken its toll on urban infrastructures and more so in its water supply capacity, which is strained to its limit at present and LSMC is no exception.

People in LSMC depend upon the well, stone spout and tap water to fulfill their needs and was the target area for water sample collection and analysis. According to the recent census the population of LSMC is 162,991 the total number of house holds is 68,922 (LMSC, 2002). It consists of number of wells; stone spouts, and tap piped line.

Collection of Water Samples for Analysis

Water samples for microbial testing were collected carefully in sterile bottles to prevent accidental contamination of the water during collection. Glass bottles used for water sampling had capacity of 10 ml and 200 ml. It was fitted with ground glass stopper or screw caps. The stopper or cap and neck were protected from contamination by a suitable cover either of paper or thin aluminum foil. Silicon rubber liners that withstand repeated sterilization by keeping more than 60 min at 160^oC in an oven. After sterilization, the bottle was not opened before the sample was collected. Samples were collected according to Cheesbrough (2000). The sterile bottle was held by the base in one hand. The other hand was used to remove the stopper and covered together. The stopper and cover were retained in the hand while the bottle was filled and then replaced together.

- **Sample Distribution**

A total of 160 samples of drinking water were randomly collected from different locations of Lalitpur Sub-Metropolitan city. All water samples were analyzed for microbial parameters to assess the drinking water quality.

Out of total 160 samples collected, well water contributed 54 (34%) and NWSC tap water contributed 42 (26%), stored water contributed 48 (30%) and stone spout contributed 16 (10%). Source wise distribution of samples showed that well water contributed the highest number of samples.

Table 2. Source wise distribution of water samples.

S.N.	Sources	No. of sample	Percentage
1	Well water	54	34
2	NWSC tap water	42	26
3	Stored water	48	30
4	Stone spouts	16	10

- **Sample Collection from Stone Spouts**

The bottle was held by base in one hand while the other hand was used to remove and replace the screw cap or the stopper. Water was collected facing the mouth of the bottle towards the opposite directions on the water current. The neck of the bottle was slightly filled upwards to let it fill completely before carefully replacing the cap. The bottle was labeled with the sample code number.

- **Sample Collection from Tube Wells**

The hand pump was operated continuously for 5 minutes. The mouth of the pump was heated preferably by means of a sprit lamp and pumped several gallons of water to waste. Sample was collected ascetically by allowing the water from the pump to flow directly into the sterile bottle and replaced the bottle cap carefully. The bottle was labeled with the sample number.

- **Sample Collection from Open Wells**

A sterile sample collecting bottle was tied on a weighed length of strong string. Stone or heavy piece of metal was used as a weight, and the bottle was attached just above the weight. The cap was removed aseptically from the bottle and the bottle was lowered into the well to a deep of about 1 meter. When no more air bubbles raised the surface, the bottle was raised out of the well and cap was carefully replaced. The sample bottle was properly labeled.

- **Sample Collection from Taps**

Any external fittings from tap were removed such as an anti-splash nozzle, or rubber tube. Outside nozzle of the tap was cleaned carefully, and the water was allowed to run for 1 minute in order to flush the tap and discharge stagnant water. The sample bottle was filled from a gentle flow of water, and replaced cap of the bottle, after which the bottle was labeled.

- **Tubes and Bottles Labeling**

The bottles and test tubes were labeled immediately before each sample was taken to allow them to be properly identified. The label includes the following information.

- Identification number
- Origin of the sample (place, type of installation etc)
- Volume of the samples
- Date and time the sample taken

This information was also recorded in a field data sheet.

- **Transportation and Preservation of Sample**

Immediately after collection, samples were transported to the laboratory of National Zoonoses and Food Hygiene Research Center (NZFHRC). They were stored in ice or refrigerated until the laboratory processing was performed.

Laboratory Processing of Water Samples for H_2S Test

- **Preparation of H_2S Media**

The concentrated medium used in the test was prepared from the above mentioned ingredients as described by Joshi *et al*. (2004). These were dissolved by stirring into distilled or no chlorinated tap water.

- **H_2S media volumes**

The H_2S test allows to estimate different degrees of contamination by using different volumes of water samples with higher or lower amounts of H_2S media impregnating the paper strips. The following are recommended variations.

Test sample volume	Volume of H_2S media added to paper strip	Recommended use
1 ml	0.5 ml	Untreated water or water with suspected contamination
10 ml	0.5 ml	Untreated water with suspected contamination
20 ml	1 ml	Treated water
100 ml	2.5 ml	Treated water in well maintained systems

- **Preparation of Paper Strips**

These are carriers for the H_2S media. The paper strips can be prepared by either placing them into test tubes or bottles and adding the H_2S media into them or by placing the paper strips on sheets of aluminum paper foil and adding the media into them. Preparation of a stock of impregnated papers was also done that can be stored for later use.

Whattman R #3 filter paper was used for our work. The size of the paper used was big enough so as to readily absorb the required H_2S culture media. The absorbent material was cut to a size that absorbs all of the media. The media and other laboratory facilities were made by NZFHRC on free of cost.

The aluminum sheet with impregnated paper strips is placed in an oven to dry of a temperature of about 70^oC for 20-25 minutes. The dried pads or strips were put into a sealed plastic bag, an envelope, or a clean glass jar and sealed from humidity for later use.

- **Sterilizing the Tubes with Paper Strips**

Before going to the field to collect water samples, test tubes or bottles with paper strips were sterilized as follows.

The caps and tubes were washed carefully and allowed to air dry (use soap, the test tube brush and rinse thoroughly with clean bottle or boiled water). For each test tube or bottle I prepared thin strips by cutting the dry impregnated strip into smaller pieces so that they can easily fit through the mouth of the test tubes or bottles. Then they were carefully placed inside the tube or bottle. A cap was loosely fitted on each tube or bottle. Each cap was covered with a small piece of tin foil to protect it from the heat (this helps the cap last longer).

The covered, capped tubes or bottles with the strips in them were placed onto a baking sheet, then into an oven preheated to 110^{o}C. The tubes/bottles were kept in the oven and allowed them to cool for 10 minutes. Then the caps were tightened and aluminum foil was removed. Sterilized tubes/bottles with dark paper strips were stored in a dark place at room temperature for up to 12 months.

- **Marking the Test Volumes for Bottles and Test Tubes**

The tubes which are going to be used to test 10 ml water samples, was added 10 ml of water (with the help of a 10ml pipette or small graduated cylinder) to one empty tube and using a permanent marking pen, marked on the tube at the maximum height of added water. Using this mark as a guide, prepared as many tubes as needed with a 10ml mark line (marking tape can also be used to do the markings).

For tubes which are going to be used to test 1ml of water samples was added 9ml of water to one empty tube and using a permanent marking pen, marked on the tube at the maximum height of the added water. Then added 1ml of water to the 9ml already added and marked at the new total level (i.e. the 10 ml level). Using these 9ml and 10ml marks as guides prepared as many tubes as needed.

Similarly, for 20 ml and 100 ml test, bottles are marked with a line or tape the appropriate volume mark (used a graduated cylinder to measure the volumes in the guide bottles)

It was important every time the samples were collected, a control sample was tested. The control can be either boiled water or bottled water. In other words: water that is known to be suitable for drinking. The water that is used as control was also used for dilution i.e. 9 ml of dilution water and 1 ml of sample.

- **Incubation**

The samples were incubated as soon as possible at a temperature between 26 to 37^{0} C for three days. If temperature decreased below 30^{0} C incubation was continued for 5 days.

- **Preparation of the Negative ("N") Control**

A small bottle of non-chlorinated water was opened just before pouring into the test tube. 10 ml of bottle non-chlorinated water was added into a tube marked (N). We used water that has been boiled for one minute (then cooled to room temperature) as control. Immediately capped the tube and placed it in the incubator with the other samples tubes. All tubes were covered with a piece of foil paper to protect them from the light of the incubator.

Colistrip and Coliplate Test of the Water Samples

Coliplate™ kit is a convenient test for quantitative measure of total coliforms and *E. coli* bacteria. The kits were provided by NZFHRC on free of cost. The test is designed to meet regulatory guidelines for surface water, recreational water, processing water and waster water. The test quantifies density of target bacteria, coliforms and *E. coli* ranging from 5 – 5000 CFU/100 ml sample without dilutions (APHA, 1989; APHA 1998).

Coliplate and colistrip are the specific enzyme coated plates prepared in Canada for culture of Coliform and *E. coli* bacteria in water.

The collected samples were proceed by filling all the cells in the plate by pouring the preserved water samples on the plate taking care that our hands do not touch the interior or its lid. When all the coliplates and colistrips were filled, they were loaded in to incubator making sure that we can observe the temperature. The incubation was done for 26 hours after which the plates were removed.

The Coliplate™ assay is based on the unique ability of coliform bacteria to utilize specialized nutrients and reagents to form a distinctive blue color, and the unique ability of *E. coli* to form a fluorescent substance under the assay conditions. Quantification of coliforms and *E. coli* is based on the principle of the most probable number (MPN) of colony forming units (CFU) per 100 ml.

Microscopic Examination of Water Borne Protozoa and Helminth Parasites

All the water samples tested by H_2S test were retested to detect parasitic eggs by using centrifugation method as well as by floatation technique.

- **Centrifugation Method**

In a clean and sterilized centrifuge test tube, sample water was taken and kept in centrifuge machine. While keeping the test tube in the machine, precaution was taken i.e. the test tube were kept parallel to each other to maintain equilibrium. Then electric power was supplied and increased simultaneously not abruptly. It was left there for 15-20min so that the heavy particles including parasitic eggs settle down.

The supernatant was pipetted out and the precipitate was taken in the slide and observed under the microscope with coverslip.

- **Floatation technique**

Two standard methods were used for this technique i.e. using saturated solution of sodium chloride (NaCl) and using zinc sulphate ($ZnSO_4$).

Saturated solution of sodium chloride (NaCl): In this method saturated solution of sodium chloride was prepared and in a sterilized test tube the above solution was poured using sterilized pipette till half of the test tube. The same test tube was filled by sample water above test tube slide was kept in such a way that the sample water touched the slide. After 10-15 minutes the slide was taken out very carefully so that the water does not spoil out. Then covering it with cover slip was observed under microscope as above.

Zinc sulphate ($ZnSO_4$): In this method the test tube was filled with sample water and little bit of $ZnSO_4$ solution was poured and kept for 10-15 minute so that the precipitate settles on the top of the test tube, where the eggs and cysts of helminthes float. After this the slide was kept on the test tube so that the slide touches the water sample. After 10-15 minutes the slide was taken out carefully and observed under the microscope as above.

The different parasitic eggs and cysts were observed under microscope.

Data Recording and Interpretation

H_2S test: The collected samples kept in the incubator were daily inspected for up to 5 days. The blackening of the indicator paper inside the test tube gave a positive result. A positive mark was then placed on the record sheet on the day the black color first appears. If no black color appears a negative mark was then placed on the record sheet.

Coliplate and colistrip: For interpretation of the results of the coliplate and colistrip tests, the number of wells showing blue color was first recorded in the recording sheets as the positive samples for coliform. Then the same samples present in the wells were observed under fluorescence under UV light. Number of wells showing peculiar shinning was recorded in the sheet for *E. coli*. Then with the help of MPN table, most probable number of the coliform and *E. coli* densities in the tested samples were calculated.

Microscopic examination: Microscopic examination of water samples were tested for helminths parasites. The results were interpreted by observing water samples under electrical microscope. First at 10X and at 40X to view clearly and finally observed under 100X to identify any helminthic parasites if present. The observed parasites were identified with the help of books having illustration and my supervisors.

Figure 1. Sample collection from open public well.

Figure 2. Sample collection from stored water.

Figure 3. Samples incubating for H2S test.

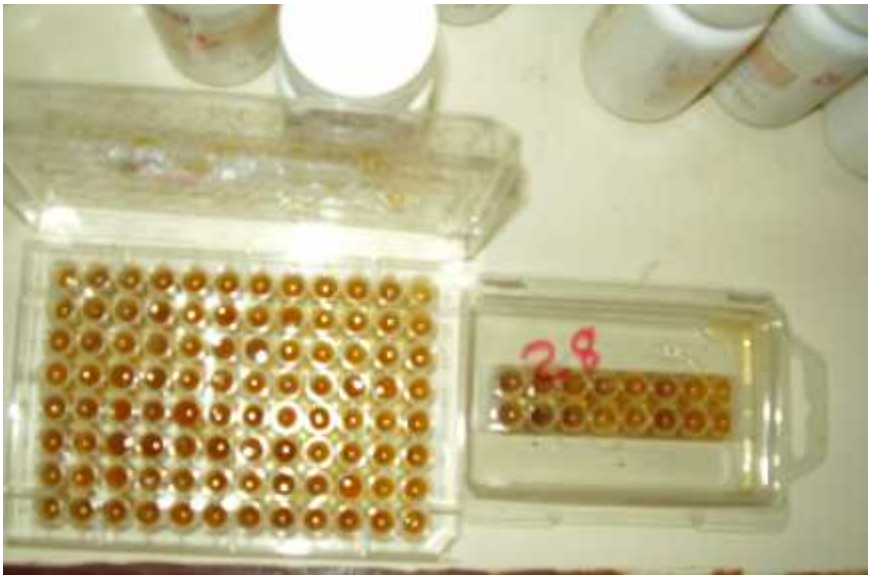

Figure 4. Samples in Coliplate and Colistrip test.

Figure 5. Laboratory analysis of water samples.

Figure 6. Examination of helminth parasites in microscope.

5. RESULTS

) Inventory of Water Sources in the Study Area

During the inventory preparation period each and every Toles, Bahals, Chowks and Gallies of the ward were visited and identified the various water sources such as stone taps, wells, NWSC tap which community people use for different purposes like drinking, washing clothes, bathing, cleaning utensils etc.

There was no any stone spout in ward number 5 and 13. There were a total of 7 public wells and 2 NWSC public taps in ward number 5. In ward number 13 there were only 2 public wells and 2 NWSC public taps. There were 1125 households in ward number 5 and around 1000 in ward number 13. In these two wards almost all house holds have NWSC tap connections but water supply is not regular. Due to the irregular and insufficient water supply in the NWSC tap people are compelled to store water and use the stored water for all purposes. People use clay pots, plastic buckets, plastic drums, metal drums, and cement tanks to store water.

People of ward no 21 and 22 were found to be extensively using natural ground water in their daily activities. In ward number 21, there were a total of 9 wells, 8 stone spouts, and 4 NWSC tap but 2 of which were permanently dried whereas in ward number 22 there were a total of 24 stone spouts, 3 public wells and 2 public NWSC taps. Total households in ward number 21 and 22 were 519 and 920 respectively. Out of which hardly 20-30% households have NWSC tap connections. Moreover, the households where the NWSC water taps connections do not have sufficient water for their all daily activities. Due to this reason the stone spouts and public wells in the wards were extensively used. Maximum stone spouts using tole in ward number 21 is of Chhayabahal and Banglamukhi of ward no. 22.

Table 3. Public water sources in ward number 5, 13, 21 and 22 of Lalitpur Sub-Metropolitan City.

Sources	Ward No. 5	Ward No. 13	Ward No. 21	Ward No. 22	Uses
Public wells	7	2	9	3	Drinking, washing, bathing cleaning etc.
Tube wells	0	0	2	0	Drinking, washing, bathing cleaning etc.
Stone spouts	0	0	8	24	Drinking, washing, bathing cleaning etc.
NWSC public tap	2	2	4	2	Drinking, washing, bathing cleaning etc.

Bacteriological Analysis of Water

Water Pollution Level Detected by H_2S Test

- **Source Wise Contamination Level of Different Water Sources**

H_2S test was done to detect the presence of H_2S producing bacteria in water. Among the various sources identified in four different wards a total of 160 samples from different sources had been tested. Out of which 57.5% water samples were found contaminated. Among the four sources stored water showed comparatively higher contamination (73%) and was followed by well water (59.3%) (Figure 7). The test result suggested that the water sources of Lalitpur sub-Metropolitan city were heavily contaminated.

Test result in various water sources identified in the study area showed on an average the water which remains stored in the households for consumption purposes were highly contaminated than the direct collected water samples from stone spouts, NWSC taps and wells. Stored water was followed by well water with 59.3% contamination. Stone spouts had lower contamination (31.3%) (Figure 7). Sample analysis in each ward clearly depicted that stored water in all wards had higher contamination (Figure 8).

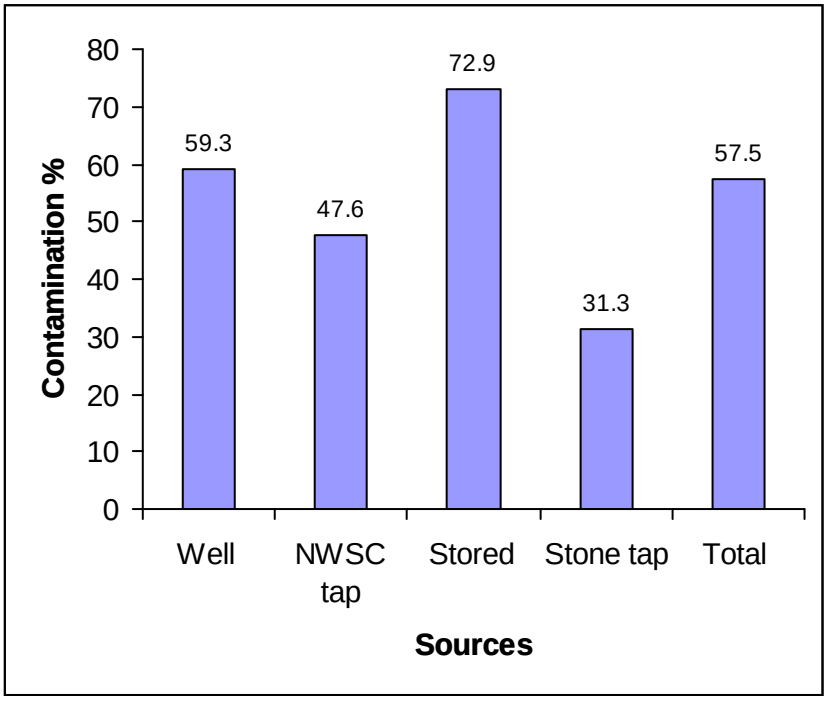

Figure 7. Source wise contamination level in Lalitpur Sub-Metropolitan City.

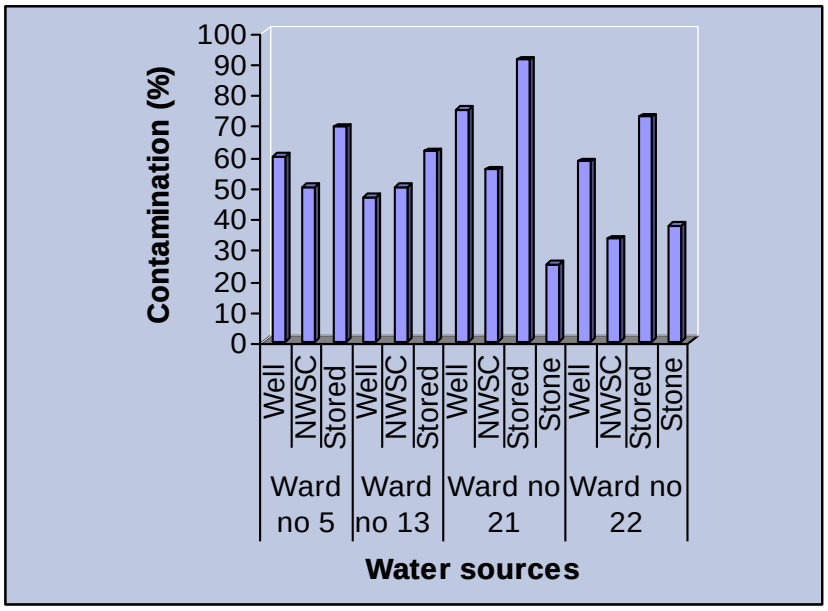

Figure 8. Contamination of water sources in different wards of Lalitpur Sub-Metropolitan City.

Samples were regularly observed for five days at every 24 hours interval. Daily observation of the samples showed that the positive percentage of the samples were comparatively higher in almost all sources on the 2nd day (Table 4). The result revealed that 29% showed positive result i.e. presence of faecal contamination on the second day while 14.4% showed positive testing on the third day and 10% on first day (Table 4) (Figure 9). The indication of positive result within the second and third day indicated high contamination of water samples with hydrogen sulphide producing bacteria of human and animal origin faecal materials and is not safe to drink unless treated.

Table 4. Source wise H_2S test observed regularly in Lalitpur Sub-Metropolitan City, Ward number 5, 13, 21, and 22.

W.N.	Sources	No. of Samples tested	1st day	%	2nd day	%	3rd day	%	4th day	%	5th day	%	Total		
													"+"	"-"	%
5	Well	15	2	13.3	3	20.0	3	20.0	1	6.7	0	0	9	6	60.0
	NWSC tap	12	1	8.3	5	41.7	0	0	0	0	0	0	6	6	50.0
	Stored	13	2	15.4	3	23.1	2	15.4	2	15.4	0	0	9	4	69.2
	Total	**40**	**5**	**12.5**	**11**	**27.5**	**5**	**12.5**	**3**	**7.5**	**0**	**0**	**24**	**16**	**60.0**
13	Well	15	2	13.3	3	20.0	1	6.7	1	6.7	0	0	7	8	46.7
	NWSC tap	12	1	8.3	2	16.7	3	25.0	0	0	0	0	6	6	50.0
	Stored	13	0	0	3	23.1	3	23.1	2	15.4	0	0	8	5	61.5
	Total	**40**	**3**	**7.5**	**8**	**20.0**	**7**	**17.5**	**3**	**7.5**	**0**	**0**	**21**	19	**52.5**
21	Well	12	1	8.3	6	50.0	2	16.7	0	0	0	0	9	3	75.0
	NWSC tap	9	1	11.1	4	44.4	0	0	0	0	0	0	5	4	55.6
	Stored	11	2	18.2	4	36.4	4	36.4	0	0	0	0	10	1	90.9
	Stone tap	8	1	12.5	1	12.5	0	0	0	0	0	0	2	6	25.0
	Total	**40**	**5**	**12.5**	**15**	**37.5**	**6**	**15.0**	**0**	**0**	**0**	**0**	**26**	14	**65.0**
22	Well	12	2	16.7	4	33.3	1	8.3	0	0	0	0	7	5	58.3
	NWSC tap	9	0	0	2	22.2	1	11.1	0	0	0	0	3	6	33.3
	Stored	11	0	0	5	45.5	3	27.3	0	0	0	0	8	3	72.7
	Stone tap	8	1	12.5	2	25.0	0	0	0	0	0	0	3	5	37.5
	Total	**40**	**3**	**7.5**	**13**	**32.5**	**5**	**12.5**	**0**	**0**	**0**	**0**	**21**	**19**	**52.5**
Total		**160**	**16**	**10.0**	**47**	**29**	**23**	**14.4**	**6**	**3.8**	**0**	**0**	**92**	**68**	**57.5**

Note: W.N. = Ward Number, "+" = Number of positive samples, and "-" = Number of negative samples

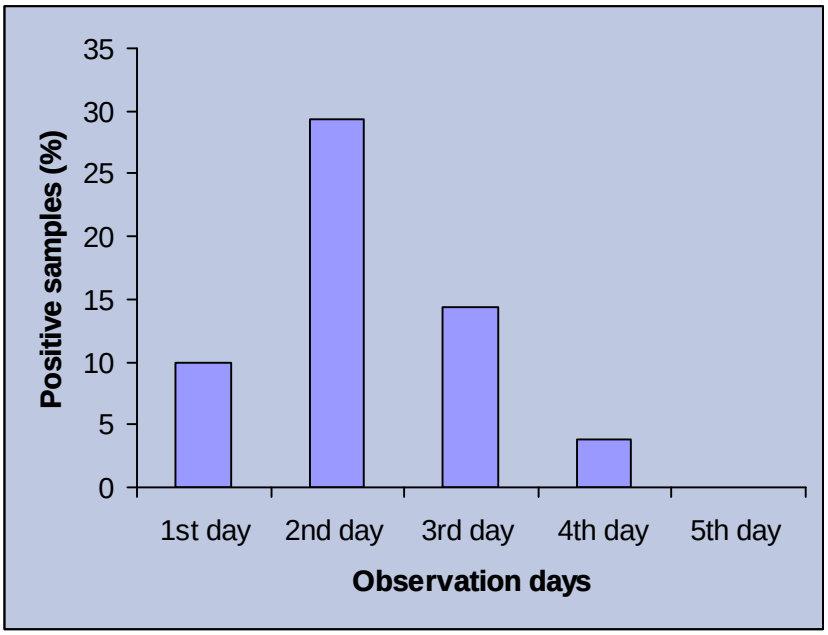

Figure 9. H_2S test result of water samples (Out of 160) observed up to 5 days.

- **Location Wise H_2S Test Result**

H_2S test result of the water samples collected from different locations showed that water contamination level was different among the locations (Table 5). Highest contamination by H_2S producing bacteria was found in ward number 21 (65%) and it was followed by ward number 5, 13, and 22 respectively (figure 10).

Water samples were collected from 28 different locations in the four different wards. Out of 28 locations Mikhabahal, Gabahal of ward number 21, Sankhamul of ward number 22 and Mathillo Kusunti of ward number 13 had 100% contamination in the tested water sampes (Table 5). On the other hand, water samples collected from Phimbahal of ward number 21 and Chobunani of ward number 22 were found less contaminated (20%).

In ward number 5 water samples from Lagankhel showed the higher contamination (75%) and AVM chowk (33%), showed lower contamination level. In ward number 13 water samples from Nakkhu had lower contamination (25%) (Table 5).

Table 5

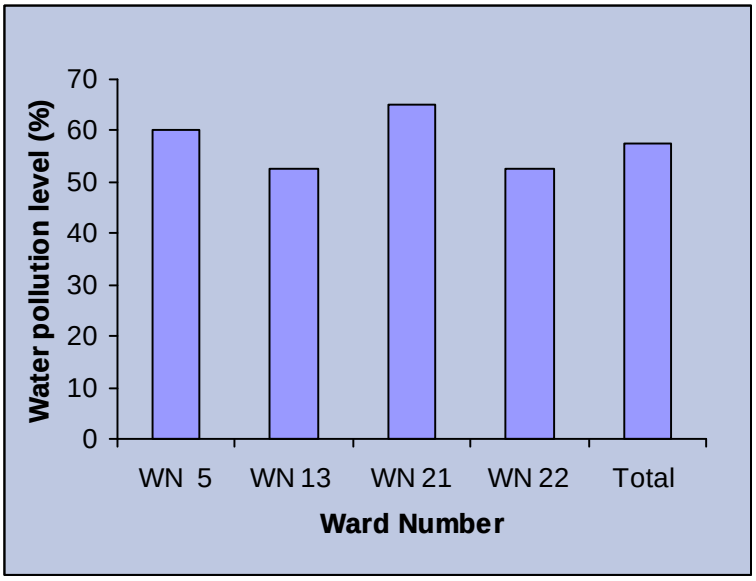

Figure 10. Water pollution level in different wards of Lalitpur Sub-Metropolitan City.

Water Pollution Level Measured by Coliplate and Colistrip Method

- **Source Wise Coliplate and Colistrip Test Result**

Positive samples obtained by H_2S test were incubated by coliplate and colistrip method to calculate the coliform and *E. coli* densities. Source wise coliform and *E. coli* densities were analyzed categorizing in to three groups (< 100, 100-500, and >500 per 100 ml of water).

The result revealed that 57.5% of the sources were found to be contaminated with Coliform bacteria and *E. coli*. Among the water samples collected from four sources (well, NWSC tap, stone spouts and stored water) stored water showed higher densities of coliform (Table 6) along with the higher H_2S test positive result (73%). On an average coliform and *E. coli* densities were <100 in more samples (Table 7).

The minimum to maximum numbers <3 – 469 *E. coli* and < 3 - >938 coliforms were detected in different water sources. Higher density of *E. coli* was found in wells. According to the WHO guidelines (see appendices table 15) the water collected from each sources were not safe for consumption.

Table 6. Bacteriological pollution level detected in different water sources of Lalitpur Sub-Metropolitan City ward no 5, 13, 21, and 22.

			Coliplate /Colistrip results	
Sources	Total samples	Positive H_2S result (%)	Coliform MPN range	*E.coli* MPN range
well	54	59.3	<3 – 307	<3 – 469
NWSC tap	42	47.6	<3 – 94	<3 – 72
stored	48	72.9	<3 - >938	<3 – 255
Stone tap	16	31.3	30 – 106	16 – 94
	160	57.5		

The results showed that most of the samples (54.3%) contained coliform <100 per 100 ml of water whereas, 33.7% samples contained coliform between 100 and 500 per 100 ml of water and only 12.0% contained >500 in 100ml of water. Similarly 62% samples had *E. coli* count <100, 32% had between 100 and 500, and only 7% had >500 in 100 ml of water (Figure 11).

Table 7. Source wise total coliform and *E. coli*.

Ward No.	**Sources**	**No. of Samples tested**	**Pollution level detected**					
			Total Coliform			**Total *E. coli***		
			<100	**100-500**	**>500**	**<100**	**100-500**	**>500**
5	Well	9	5	4	0	6	2	1
	NWSC tap	6	3	3	0	4	2	0
	Stored	9	2	4	3	5	3	1
	Total	**24**	**10**	**11**	**3**	**15**	**7**	**2**
13	Well	7	4	2	1	5	2	0
	NWSC tap	6	5	1	0	5	1	0
	Stored	8	5	2	1	6	1	1
	Total	**21**	**14**	**5**	**2**	**16**	**4**	**1**
21	Well	9	4	4	1	3	6	0
	NWSC tap	5	2	3	0	5	0	0
	Stored	10	6	2	2	5	3	2
	Stone tap	2	2	0	0	2	0	0
	Total	**26**	**14**	**9**	**3**	**15**	**9**	**2**
22	Well	7	3	2	2	3	3	1
	NWSC tap	3	3	0	0	2	1	0
	Stored	8	4	3	1	5	3	0
	Stone tap	3	2	1	0	1	2	0
	Total	**21**	**12**	**6**	**3**	**11**	**9**	**1**
Total		**92**	**50 (54.3%)**	**31 (33.7%)**	**11 (12%)**	**57 (62%)**	**29 (31.5%)**	**6 (6.5%)**

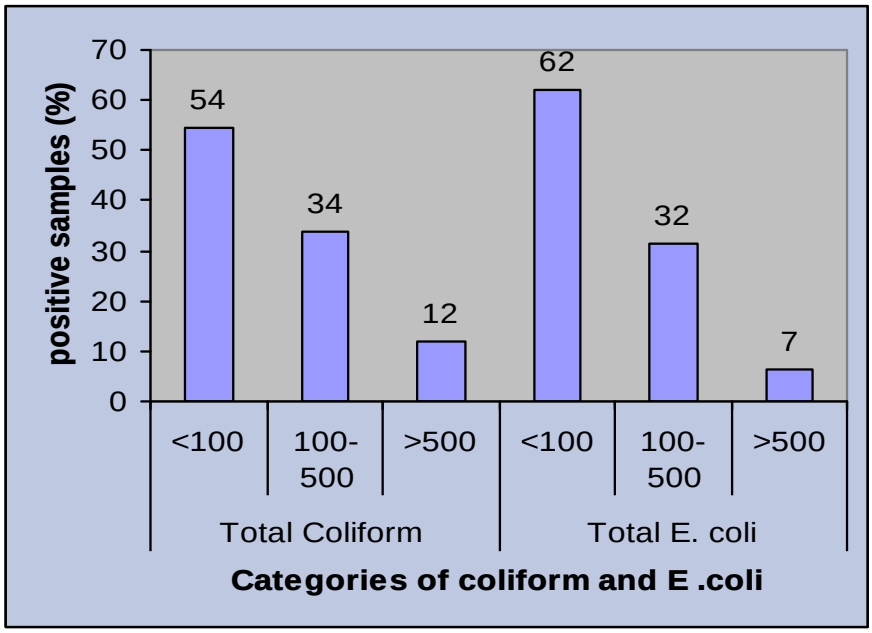

Figure 11. Coliform count and *E. coli* in different categories.

- **Location Wise Coliform and *E. coli* Count in Water Samples**

Location wise coliform and *E. coli* level in different water sources were analyzed categorizing into two groups (<100 and >100 per 100 ml of water) i.e. tolerable and non tolerable. First one can be sued after simple treatment while next one needs to be properly treated before consumption.

The bacteria densities varied in the different locations and sources (Table 8). Coliform and *E. coli* density count result revealed that in ward number 5 comparatively more water samples (58%) showed coliform densities >100 (Table 9). On the other hand more water samples (47.6%) from ward no 22 showed *E. coli* densities >100 in 100 ml. Maximum Coliform and *E. coli* densities (ranged from <3 - >938 and <3 – 469) were found in ward number 21(Table 10).

Table 8

Table 9. Densities of Coliform and *E. coli* in water samples of ward no 5, 13, 21, and 22 of Lalitpur Sub-Metropolitan city.

Ward No	Total tested samples	Percentage of water samples showing positive results			
		Total coliform		Total *E. coli*	
		<100	>100	<100	>100
5	24	41.7	58.3	62.5	37.5
13	21	66.7	33.3	76.2	23.8
21	26	53.8	46.2	57.7	42.3
22	21	61.9	33.3	52.4	47.6

Table 10. Coliform and *E. coli* MPN range in different water sources of ward number 5, 13, 21, and 22.

Ward No.	Total Samples	Positive samples	%	Coliplate /Colistrip results	
				Coliform MPN range	*E. coli* MPN range
5	40	24	60	<3 – 307	<3 – 255
13	40	21	52.5	<3 – 307	<3 – 119
21	40	26	65	<3 - >938	<3 – 469
22	40	21	52.5	<3 – 280	<3 – 119
	160	92	57.5		

J Microscopic Examination of Parasites

Microscopic examination of water samples from different sources (wells, NWSC taps, Stone spouts and stored water) were examined during the study period for the detection of Helminths and Protozoan parasites which are of medical, veterinary and zoonotic importance. Two methods were adopted i.e. floatation and centrifugation method. While doing examination various types of eggs and adult worms of helminths were found (Table 11), which may cause sever illness to human and animals while injested along with the water without treatment. Highest number of parasitic contamination was found in water samples collected from wells (13%) (Table 12) whereas, NWSC tap had the lower parasitic contamination (5%). The detected parasites were *Ascaris* spp, *Trichuris* spp, *Taenia solium* and *Ancylostoma* spp (Table 13). Eggs of *Ancylostoma* spp were found comparatively higher than other species. Higher number of eggs was observed by centrifugation method than floatation method (Table 12).

Table 11

Table 12. Source wise parasitic contamination of different water sources by floatation and centrifugation method.

Water sources	Total tested samples	Positive result					
		Floatation Method		Centrifugation method			
		No. of positive samples	%	No. of positive samples	%	Total positive samples	%
Well	54	3	6	4	7	7	13.0
NWSC tap	42	1	2	1	2	2	4.8
Stored water	48	1	2	2	4	3	6.3
Stone tap	16	1	6	1	6	2	12.5
Total	160	6	4	8	5	14	8.8

Table 13. Different parasitic eggs/worms detected in water samples of different sources.

S.N.	Sources	Total tested samples	Positive samples	Parasites
1	Well	54	7	Eggs of *Ascaris* spp, *Trichuris* spp, *Taenia solium* and Unidentified nematodes
2	NWSC tap	42	2	Unidentified nematodes
3	Stored water	48	3	Unidentified nematodes
4	Stone Spouts	16	2	Eggs of *Ancylostoma* spp

J Seasonal Variation of Water Pollution

Water samples were observed for two seasons i.e winter and rainy seasons. In winter season only 2 samples (out of 160 samples) collected from well of Lagankhel of ward number 5 were found positive by H_2S test and coliform and *E. coli* count in these 2 positive samples ranges from <3 to 65 and <16 to 52 respectively whereas in rainy season 92 samples (out of 160 samples) showed positive result.

The coliform and *E. coli* count in rainy season ranged from <3 to >938 and <3 to 469 (Table 14). The study pointed out the clear linkage between the season and the level of coliform densities, indicating lower degree of pollution during winter season.

Table 14. Variation in water samples collected during winter and rainy season.

	Winter season	Rainy season
Number of samples	160	160
Minimum coliform count/100 ml	<3	<3
Maximum coliform count/100 ml	65	>938
% of samples with coliform count	1.3	57.5
Minimum *E. coli* count/100 ml	<16	<3
Maximum *E. coli* count/100 ml	52	469

Figure 12. Test tubes with positive and negative H_2S test result.

Figure 13. Free living nematode detected in water samples.

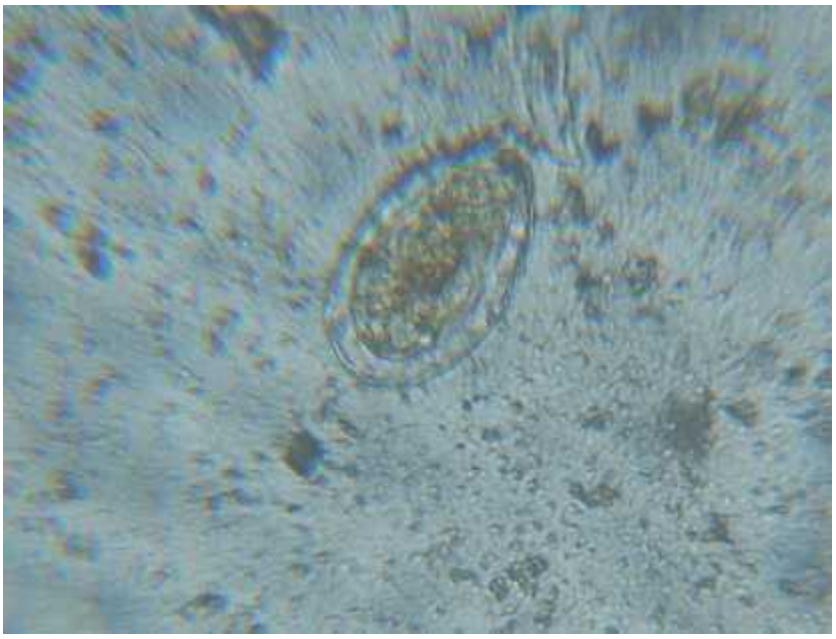

Figure 14. Egg of *Ascaris* spp detected in water samples.

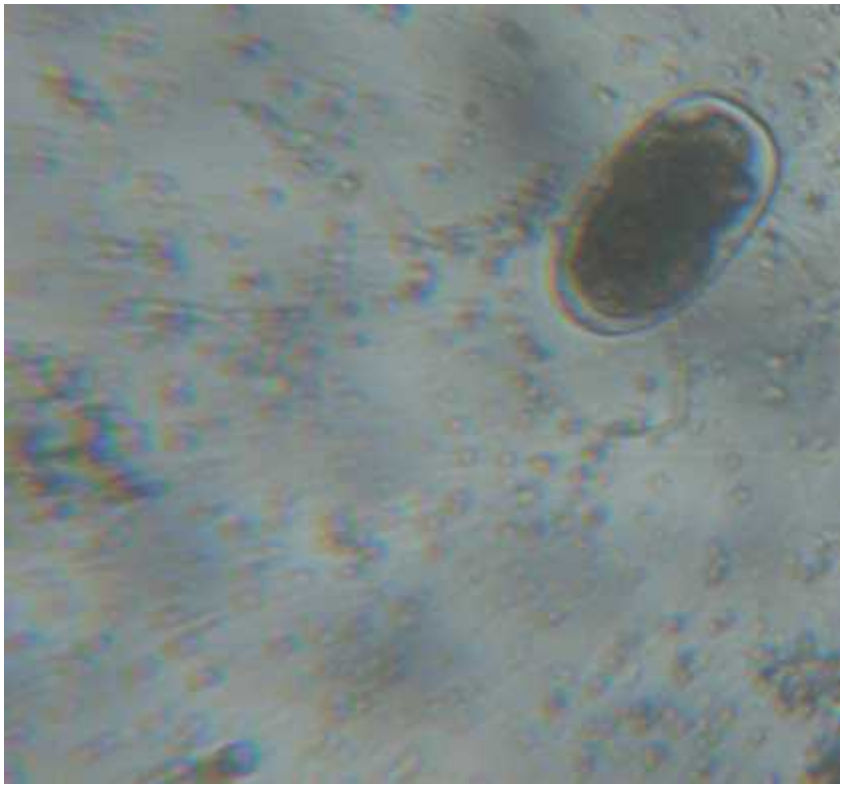

Figure 15. Egg of *Ancylostoma* spp detected in water samples.

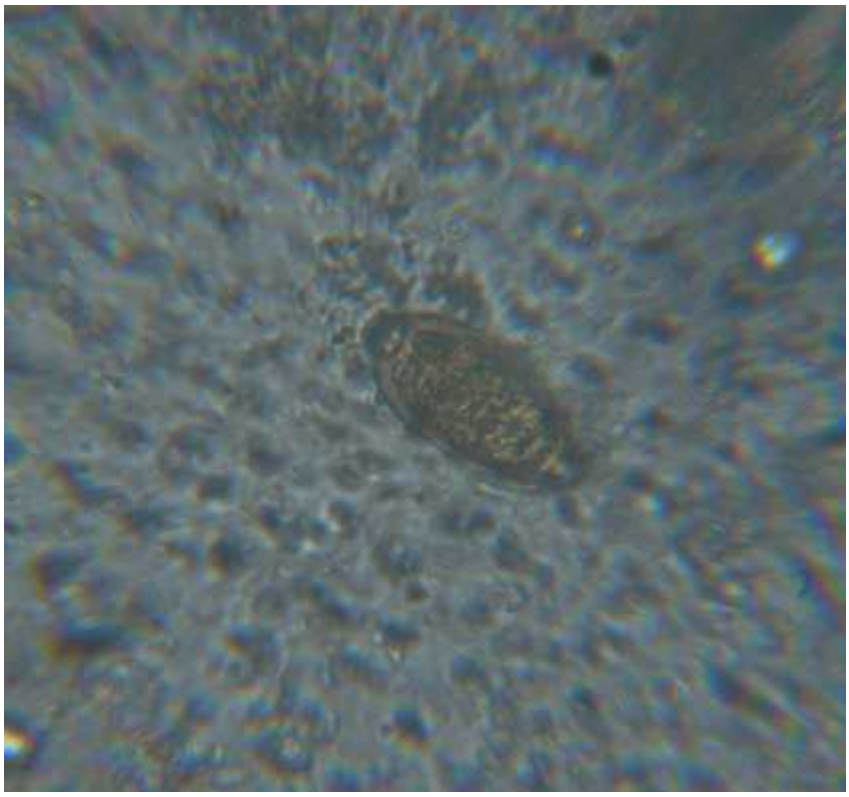

Figure 16. Egg of *Trichuris* spp detected in water samples.

6. DISCUSSION

The study was carried out to assess the microbiological quality of drinking water used by the people in Lalitpur Sub-Metropolitan city. Communities have access to four types of water sources (tap, well, stone spout, and pond) for domestic purposes. People in the urban area of Lalitpur mainly depend upon the well, stone spout, and tap water to fulfill their needs.

Department of Drinking water supply HMG/N and Nepal Water Supply Corporation (NWSC) are responsible for supplying drinking water and sewerage service to the municipal areas of Kathmandu. The existing water supply service is not equitable. Some consumers of low-lying areas and near transmission mains enjoy 24 hours supply, whereas most of consumers receive only a few hours' supplies in a day moreover many received water in an alternate days or only 2-3 days per week or sometimes not supplied for weeks too. Consumer survey report by Tiwari (1999) reported about only 34% of total have either good or sufficient water flow. Thus people in Lalitpur are looking for alternate means of water though they prefer to use piped water. Therefore, the main objective of the study was evaluation of quality of water from different sources (tap, stone spout, and well) in Lalitpur.

The previous studies carried out to-date showed that the water quality is deteriorating with time in all types of sources. All studies carried out by Sharma (1978), Sharma (1986), Adhikari *et al.* (1986), ENPHO/DISVI (1995), Maharjan (1998), Prasai (2002), and Diwakar (2007) indicates that the public water supply is far from satisfactory in almost all localities. Sharma (1978) found only 50% samples contaminated with faecal material in Kathmandu but in a follow up study in 1986 Sharma found that the level of coliform contamination had significantly increased 85% in between nine years. Adhikari *et al*. (1986) found 88% of the samples unsatisfactory. Likewise, CEDA (1989) recorded that all samples were contaminated with faecal matter and none of the tap and ground water sources were safe for drinking. Pradhananga *et al*. (1993) found average coliform count ranging from 8col/100 ml to 88col/100 ml was recorded in six stone spout around Pashupati area. Maharjan (1998) found that 85.6% of samples randomly collected from shallow pumps, shallow wells, stone spout dug well in urban area of Patan city contained total coliforms and 68.6% contained faecal coliforms. Recovery of *Enterobactoer* spp was found maximum followed by *Escherichia coli*> *Citrobacter* spp> *Salmonella* spp and others. Similarly, Prasai (2002) showed that the majority of water samples (82.65%) crossed the guideline value as prescribed by WHO and only 17.39% samples were safe. Sharma (1993) examined bacteriological quality of the potable water of different urban and rural areas of Nepal and reported that maximum densities of coliforms and faecal coliforms were detected in Kathmandu, then in Hetauda, followed by Birgunj, Pokhara and Biratnagar. This study indicates urban area is heavily contaminated by coliform bacteria than in rural area.

In consistent with the previous studies in this study also, no water sources were free from contamination. Almost all water sources wells, stone spouts, also NWSC taps were found contaminated. A total of 57.5% water samples were found contaminated. Coliform densities ranged from <3 - >938.

The existing studies indicated that the quality of drinking water in most of the places in Kathmandu is not acceptable. Gyawali (2002) also reported 90% of water samples contaminated in her study. Presence of *E. coli* in drinking water is an indication of faecal matter contamination and therefore there is always risk of presence of pathogens. WHO guide line recommends that *E. coli* should not be detected in drinking water. Among the different sources, deep wells and shallow wells are least contaminated and dug wells are the most contaminated in the study carried by ENPHO (1999).

ENPHO/DISVI (1990b) carried out microbiological tests of drinking water in seven rural areas of Ilam and found that water samples from springs aquifers and river had unacceptable levels of faecal coliform bacteria's ranging from 2 to 2400 cell/100 ml. In other studies ENPHO/DISVI (1990a) investigated the bacteriological quality of reports from 21 localities which were found to be faecally contaminated. And in another study carried out in Baluwa and Gokarna VDC, out of 16 samples analyzed enteric, pathogenic bacteria *E. coli* was detected from all with total coliforms count ranged form 150 to 1100 cells/100 ml. Among them 30% samples contained an average coliform count of more than 100 cells/100 ml. similar result was obtained in our study too.

In this result among the various sources, stored water showed higher contamination. This might be due to the dirty storage facility. People store water in any type of container without proper cleaning and disinfection. Due to the irregular and insufficient water supply from NWSC tap people in Lalitpur are compelled to store for a long period for their daily needs. In previous studies in which paired water samples from individual water sources and household storage containers were compared, the results were similar; faecal coliform concentration were generally and sometime dramatically, higher in stored water than in source water. Faecal (thermotolerant) coliform bacteria may not by ideal indicators for faecal contamination. In some field studies, enteropathogens were identified in stored water. In Thailand, enterotoxigenic *E. coli* was recovered from a drinking water jar used to store rain water. In Bangladesh, toxigenic *Vibrio cholerae* (01) was recorded from stored drinking water collected from safe tube wells and in Calcutta Deb *et al*. (1982) isolated *V. cholerae* (01) from stored water in 9% of household of Cholera patients and in 2% of control household. Deb *et al*. observed that people generally took stored water from the open bucket by dipping, thus resulting in contamination of otherwise safe water by their infected fingers.

During a Cholera epidemic in Bahrain in 1981, investigators identified *V. cholerae* (01) in stored household drinking water. Similarly in Myanmar, toxigenic *E. coli* was identified in two of 40 water samples form household's storage vessels but in none of 20 samples collected on the same day from the direct sources. In Egypt, two parasitic pathogens, *Strongyloides* and *Ascaris*, were isolated from 10% to 15% of water samples collected earthenware household storage vessels, but no pathogens were identified in source water samples. These studies indicates that contamination with pathogens as well as indicator organisms occurs during home water storage this correlated to our present study which showed greater contamination in stored water than source water.

The risk of diarrhoeal diseases due to contamination of drinking water during household storage was noticed in surveys conducted by the WHO in the 1960's. The WHO team observed that drinking water taken from piped supply, stored for cooling in earthen jars was, without exception faecally contaminated.

Almost all water sources were found to be microbiologically contaminated might be due to infiltration of various kind of pollutants, originating from many sources such as oil spills from refineries, toxic waste from chemical plants, biological waste from hospital and leachates from peri-urban landfills resulting in plumes of pollutants traveling in the ground water and contamination underground environment. Domestic waste water, industrial waste, increase in use of agro-chemical, hazardous waste disposal sites add ground water contamination. All the natural sources such as stone spouts, wells and tube wells are neither treated nor protected properly. The result highlights the deteriorating water quality which may contribute different types of water borne diseases at any time. Deteriorating water quality impacts health and economy of a country. A range of health sector interventions, environmental pollution, unsafe water, poor sanitation practices, malnutrition, behavioral attitudes, illiteracy, climate condition and poverty continue to be responsible for the persistence of pre-transition diseases. Unavailability of chlorine in the form of gas and poor condition of drinking water supply pipes are among the major causes for degradation of public tap water quality. Other causes may be due to the use of unrepaired old pipeline systems for distribution, parallel arrangement of drinking water pipeline with that of the drainage system and irregular supply of the drinking water in the pipeline, the failure of the disinfection of the raw water at treatment plant or to the infiltration contaminated sewage through cross connection, leakage points and back siphonage. In piped supplies, discontinuity increase the likelihood of contamination as the risk of back siphonage into the distribution network is increased when piped are at lower pressure than the surrounding soil, when often contains leaked out effluent from leaking sewers.

Natural water sources, such as ground water sources, like the tube wells and deep wells including surface water sources like stone taps, are neither treated nor protected properly. In some locations some of the personal tube wells remain protected, closed from outside but the waster drainage pipe is not so far from the wells and tube wells. Physical characteristics of most of the well water are not so clear and found contaminated with the soil and sand particles, and transparency is also not very clear. There are a few wells found in some locations which look clear and have no odour but are bacteriologically not safe. Only the tap water supplied from Nepal Drinking Water Supply Corporation is treated. The problem is in the distribution system where most of the drinking water pipelines are bought parallel to that of the waste water drainage pipelines. Hence during the rainy season most of the drainage pipelines have broken down and contaminate the drinking water. The principal reason of the drinking water contamination are due to the use of unrepaired old pipeline systems for distribution, Parallel arrangement of the drinking water pipeline with that of the drainage system and irregular supply of the drinking water in the pipeline. When drinking water is not supplied in the pipeline they remain filled up with the air. If there is waste water around them very easily the waste water is socked into the drinking water pipeline and at the time of drinking water supply the water becomes contaminated, although they are previously treated.

So most of the community people complain about faecal matters seen in the drinking water at times. All the information regarding the water sources and supply systems indicate that the possibility of water getting microbiological or faecal contamination is high.

This result slightly contradicts with the previous report by Joshi and Maharjan (2003). They reported that water supplied from Nepal Drinking Water Supply Corporation had very few contaminations and was safe for drinking purposes. In this study water samples collected from the NWSC tap were also found bacteriologically contaminated and coliform densities ranged form <3-92.

In this study microbial contamination of drinking water is varied with the season. In spring season only 2 out of 160 samples were contaminated whereas in rainy season 92 out of 160 samples were contaminated. The result pointed out the clear linkage between seasons and level of coliform densities, indicating lower degree of pollution during winter in consistent with the result reported by ENPHO/DISVI (1995). In the report by ENPHO/DISVI (1995) lower densities of coliform were reported in winter (Jan-March) while from April it increased, reaching the maximum level of contamination in June. The result suggested that in rainy season the volume of water bodies and blow of surface water source increases and water bodies become contaminated due to seepage or sewage, waster water, and runoff from adjacent land. Simultaneously total bacterial and coliform count increases along with the nature of the population.

Sharma and Bhardwaj (2000) studied the bacteriological contamination of the water samples of Kalayatji village pond of Bikaner (Rajasthan). Bacteriological analysis of water revealed that the contaminated nature of the pond and bacterial density varied according to the season. Highest values were observed in monsoon. It was positively correlated with the water temperature and rainfall.

Water samples were examined for protozoan and helminthes parasites. Floatation and Centrifugation methods were applied for the test. The microscopic examination result indicated that water samples were contaminated with several parasites. Previous studies also reported the presence of parasites in drinking water. Karanis, (2000) investigated the parasitic zoonotic diseases and its agents in drinking water. He stated that the human and veterinary important parasites of subkingdom of protozoans and helminthes infect humans and animals by injection of parasites in contaminated water. The parasites are excreted from the body of infected humans, livestock, zoo animals, companion animals or wild animals in the faeces. Recreational water, agricultural practice and wild animals serve as vehicles of transmission of the parasites in the water supplies. Gibson *et al*. (1998) investigated the occurrence of two pathogenic protozoa, *Cryptosporidium* and *Giardia, in* an urban stream. *Cryptosporidium* oocysts and *Giardia* cyst were commonly observed in the urban stream during dry weather conditions, with concentrations of 5-105 oocysts/100L and 13-6579 cysts/100L respectively. A study conducted by Thapa (2002) in Kathmandu Metropolitan City, ward no 20 reported the presence of *Ascaris* spp, *Cryptosporidium* spp, *Capilaria* spp, *Toxocara* spp, *Toxoplasma* spp, *Taenia* spp in drinking water sources.

Similarly Gyawali (2002) also reported several parasites i.e. *Ascaris* spp, *Cryptosporidium* spp, *Capilaria* spp, *Toxoplasma* spp, *Taenia* spp, *Gnathostoma* spp, *Entamoeba* spp, *Trichuris* spp, *Fasciola* spp and *Enterobius* spp etc. While in the present study eggs of *Ancylostoma* spp, *Ascaris* and *Trichuris* spp and some unidentified nematodes were observed.

The detection of faecal coliform, *E. coli* in samples points towards the faecal contamination of the natural sources of water. Dirty storage facility is mainly responsible for the contamination in stored water. People consume these waters with great pleasure without realizing that they are subjecting them to plethora of water borne diseases. In future, such studies must be regularly performed and control measures in form of chlorination, protection of natural sources and provision of adequate piped water supply must be undertaken by public health authorities so as to reduce morbidity and mortality caused by epidemics of water borne diseases.

7. RECOMMENDATIONS

The study revealed a picture of the drinking water quality situation of Lalitpur Sub-Metropolitan City. Bacteriological pollution in all kinds of drinking water sources could be one of the major factors of the outbreak of water borne epidemics. So, preventive measures are urgently needed to control the occurrence of such un-pleasurable incidence. There could be several long term programs like rehabilitation of treatment plants, restoring the pipe network, find out alternative water sources and provide piped water in all the communities.

Besides these several short terms, instant and feasible programs could be brought into action for the betterment of the water quality in the existing situation. Some recommendations made from the study are as follows.

- Regular water quality monitoring should be carried out to check up the degree of pollution and its causes.
- Public awareness programs for water quality and water related disease should be launched in the communities through local NGOs and INGOs and from government sector.
- People should be trained about personal hygiene and simple water treatment methods such as boiling, filtration, SODIS etc.
- Protection of traditional sources like stone spouts, dug wells should be done. If it cannot be done people should be informed about the quality of water from such sources.
- Capacity building at the community level (water users committee) will help to address water quality problems and to raise public awareness.
- Chlorination and disinfection of drinking water both in community levels and for individual households should be strictly applied.
- Higher number of samples from different sources should be tested regularly and additional research is needed on microbial evolutionary ecology to address long term public health issues.
- Proper storage system and treatment of water must be taught in community level as well as household level.

8. REFERENCES CITED

Abdel, M.O., (2001). Bacteriological and Chemical Evaluation of Drinking Water in Qulubyia Egypt. Egyptian Journal of Microbiology; 35(3): 343-354.

Adhikari, R.K., Rai S.K., Pokharel, B.M., and Khadka, J.B., (1986). Bacteriological Study of Drinking Water of Kathmandu Valley, Journal of Institute of Medicine; 8(3): 373-376.

Albala, D.M.,and Taylor, D., (1985).Water Quality and Diarrhoeal Diseases in Rural Community of Eastern Nepal. J. Inst. Med ; 7: 35-42.

Al-Thani, R.F., (2003). Coliform Bacteria in the Waste Water Ponds of Doha City, Qatar. Quatar University Science Journal; 23: 99-107.

APHA, (1989). Standard Methods for the Examination of Water and Wastewater. Physical and Aggregate Properties. (17th ed.). American Public Health Association, Washington DC. Pp 3-76.

APHA, (1998). Standard Methods for the Examination of Water and Wastewater. Prepared and published jointly by American Public Health Association, American Water Works Association, and Water Environment Federation. (20th ed.). American Public Health Association, Washington DC. Pp 9-56.

Arvanitidou, M., Kanellou, K., and Vagiona, D.G., (2005). Diversity of *Salmonella* spp in Northern Greek Reverse and Their Correlation to Faecal Pollution Indicators. Environmental Research; 99(2): 278-284.

Bajracharya, A.M., (2007). Study of Drinking Water Quality of Kathmandu Metropolitan Areas and Evaluation of Antibacterial Property of Some Medicinal Plants against Isolated Enteric Bacteria. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Bilgrami, K.S. and Kumar, S., (1998). Bacteriological Contamination in Water of the River Ganga and Its Risk to Human Health. Journal of Environmental Health Research; 9(1).

Borrego, J.J. and Figueras, M.J., (1997). Microbiological Quality of Natural Water. Microbioligia (Madrib); 13(4).

Bottino, A., Thapa, A., Scantoline, A., Ferino, B., Sharma, S., and Pradhananga, M.T., (1999). Pollution in the Water Supply System of Kathmandu City. Journal of Nepal Chemical Society; 10: 33-44.

Bovier, E.K., (1978). First Report on Millipore Bacteriological Water Tests, Rural Water Supply Noted "A" Gandaki Zone. LDD. UNICEF/SATA, Pokhara, Nepal.

CEDA, (1989). A Study on the Environmental Problems Due To Urbanization in Some Selected Nagar Panchayat of Nepal. Report submitted to UNDP/ Kathmandu. Centre for Economic Development and Administration, Tribhuvan University, Kathmandu, Nepal.

Cheesebrough, M., (2000). Water Related Diseases and Testing of Water Supplies. In: District Laboratory Practice in Tropical Countries Part (2). Low Price Edition 2000, Cambridge University Press. Pp 143-157.

Clang, J.L., (2000). Drinking Water Quality Crisis Occurred in Sydney Australia, From July through September 1998. American Water Works Association Journal; 92(3): 55-66.

Craun, G., Rebecca, F., Calderan, L., and Craun, M.F., (2005). Outbreaks Associated with Recreational Water in the United States. International Journal of Environmental Health Research; 15(4): 243-262.

Deb, B.C., Sirkar, B.K., Sengupta, S.P., (1982). Intrafamilial Transmission of *Vibrio cholera* Biotype Eltor in Calcutta Slums. Indian J. Med. Res.; 76:814-819.

Dhaubadel, M.S., (1992). Study in Drinking Water Quality of Bhaktapur. A dissertation submitted to the Central Department of Zoology in partial fulfillment of the requirements for the award of the degree of Master of Science in zoology, Tribhuvan University, Kirtipur, Nepal.

DISVI, (1990). Water Quality Monitoring of Kathmandu City Water Supply. Environment and Public Health Organization (ENPHO) and DISVI International Co-operation, Kathmandu, Nepal.

Diwakar, J., (2001). Assessment of Drinking Water Quality of Bhaktapur Municipality. A dissertation submitted to the Central Department of Zoology in partial fulfillment of the requirements for the award of the degree of Master of Science in zoology, Tribhuvan University, Kirtipur, Nepal.

do Amaral, L., Augusto, A.N., Filho, O.D.R., Junior, F.L. Ferreira, A., and Barros, L.S.S., (2003). Drinking Water in Rural Farms as a Risk Factor to Human Health. Revista De Saudi Publica; 37(4): 510-514.

DoHS, (1998). Annual Report (1997/98). DoHS, Kathmandu, Nepal.

DoHS, (2005). Annual Report 2003/2004. Department of Health Services, Kathmandu, Nepal.

ENPHO, (2000). Kathmandu City Looking Through the Eyes of ENPHO, Environment and Public Health Organization A Decade, 10th Anniversary Souvenir. Kathmandu, Nepal.

ENPHO, (2001). A Diagnostic Survey Report on Drinking Water Quality and Sanitation in the UNICF's Project Area Kavre, Parsa and Chitwan, ENPHO, Kathmandu, Nepal.

ENPHO, (2005a). Ground Water Quality Surveillance in Kathmandu and Lalitpur Municipality Area, JICA/MPPW/ENPHO, Kathmandu, Nepal.

ENPHO, (2005b). Final report on Assessment of Water Quality of NWSC in Hilly Regions. Environmental and Public Health Organization, Kathmandu, Nepal.

ENPHO, (2005c). Fifteen Years of Enabling Communities Become Ecofriendly. Annual Report 2005, Fourteenth General Assembly. ENPHO, Kathmandu, Nepal. Pp 5-13.

ENPHO,(1999). Monitoring of Ground Water Quality in the Kathmandu Valley. Environment and public Health Organisation, Kathmandu, Nepal.

ENPHO/DISVI, (1990). Water Quality Testing in Ilam, Environment and Public Health Organization and DISVI International Co-operation, Kathmandu, Nepal.

ENPHO/DISVI, (1991). A Case Study on Gastroenteritis Epidemic in the Mid-Western Region of Nepal (Rukum and Dang). ENPHO/DSVI International Co-operation, Kathmandu, Nepal.

ENPHO/DISVI, (1991). Water Quality Testing in Siraha. Environment and Public Health Organization and DISVI International Co-operation, Kathmandu, Nepal.

ENPHO/DISVI, (1992). Monitoring of Kathmandu City Water Supply With Reference to Chlorination and Microbiological Quality. Environmental and Public Health Organization and DISVI International Co-operation, Kathmandu, Nepal.

ENPHO/DISVI, (1995). Biological Quality of Drinking Water in Kathmandu City, A review of ENPHO/DISVI Reports, 1988-1992, Environmental and Public Health Organization, Kathmandu, Nepal.

ENPHO/DISVI, (2000). Water Quality of the stone spouts (Kathmandu city) 1989-1990, Environment and Public Health Organization and DISVI International Co-operation, Kathmandu, Nepal.

Francy, D.S., Helsel, D.R., and Nally, R.A., (2000). Occurrence and Distribution of Microbiological Indicators in Ground Water and Stream Water. Water Environment Research; 72(2): 152-161.

Ghimire, M.K., (1996). Ground water quality analysis in Patan area. A dissertation submitted to the Central Department of Mateorology in partial fulfillment of the requirements for the award of Masters Degree in mateorology, Tribhuvan University, Kirtipur, Nepal.

Giatti, L.L, Rocha, A.A., dos Santos, F. A., Bitencourt, S. C. and Pieroni, S. R. D., (2004). Basic Sanitary Conditions in Iporanga, Sao Paulo State, Brazil. Revista De Saude Publica; 38(4): 571-577.

Gibson, C.J., (1998). Combined Sewer Outflows: A Source of *Cryptosporidium* and *Giardia*. Biological Abstract.

Global Resources Institute and the International Buddhist Society, (1999). Water Survey, Water Testing, and Testing of Solar Disinfecion in Lumbini, Nepal, Global Resources Institute. Eugene, Oregan 97440, USA.

Godfrey, S., Timo, F. and Smith, M.., (2005), Relationship between Rainfall and Microbiological Contamination of Shallow Ground Water in Northern Mozambiqua. Water SA (Pretoria); 31(4):609-614.

Gorakhapatra, (1998). Outbreaks of Gastero-enteritis and Fever. Gorakhapatra Daily, May 18th 1998.

Grover, P.S., and Thakur, K., (2001). Shimla Drinking Water- A Bacteriological Analysis. The Journal of Communicable Diseases; 33(1): 44-52.

Gyawali, G., (2002). Bacteriological and Helminthological Assessment of the Drinking Water Quality of Ward 19. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Haas, C.N., (2000). Epidemiology, Microbiology and Risk Assessment of Water Borne Pathogens including *Cryptosporidium*. Journal of Food Protection; 63(6): 827-831.

Horman, A., Hanen-Finne, R.R., Maunula, L., Bonsdorft, C.H.V. Torvela, N., Heikinheimo, A. and Hanninen, M.L., (2004). *Camphylobacter* spp, *Giardia* spp, *Cryptosporidium* spp, Noroviruses and Indicator Organisms in Surface Water in South Western Finland. Applied and Environmental Microbiology; 70(1): 87-95.

ICIMOD/MoEST/UNEP, (2007). Kathmandu Valley Environmental Outlook. ICIMOD/MoEST/UNEP. Hill Side Press (P) Ltd., Kathmandu, Nepal. Pp 1-10, 57-72.

Jacobson, G., (1996). Urban groundwater database. AGSO Report, Australia, Availability: http://www.clw.csiro.au/UGD/DB/Kathmandu/Kathmandu.html. (Cited on 25 June 08).

Jarmey, S.C., (2000). A Novel Method for Detection of Viable *Giardia* cyst in Water Samples. Water Research.

Jayana, B.L., (2007). Assessment of Drinking Water Quality of Madhyapur Thimi and Study of Antibacterial Effect of Lime Juice Against Bacterial Isolates. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Joshi, D.D., and Maharjan, M., (2003). Urban Ecosystems and Health in Kathmandu: Community Based Biological Assessment of Drinking Water Sources In: Martin J. B, V. M. Suresh and T.V. Kumarnan, eds., Proceedings of the Third International Conference on Environment and Health, Chennai, India. 15-17 December, 2003. Chennai: Department of Geography, University of Madras and Faculty of Environmental Studies, York University. Pp 186-195.

Joshi, D.D., Sanchez, A., Dutka B., Karki, M., and Maharjan, M., (2004). Community Based Water Quality Monitoring and Drinking Water Management Manual. National Zoonosis and Food Hygiene Research Centre (NZFHRC), Kathmandu, Nepal.

Joshi, D.D., Thapa, S., Gyawali, L., and Karanis, P., (2007). Assessment of Water Borne Helminthic Parasites in Drinking Water Sources of Kathmandu City, Nepal. Paper Presented in Joint International Tropical Medicine Meeting on Health Security in the Tropics held on 29 – 30 November 2007 at Geneva, Canada.

Joshi, D.R. and Baral, M.P., (2004). Chemical and Microbiological Quality of Kathmandu Valley. Abstracts of the 4th National Conferecne on Science and Technology. March 23-26, 252 pages.

Joshi, P.L., (1993). Feasibility Study of Rajkulo ; Rehabilitation of Patan's Traditional Water Supply Network, Main Report; I: 10-56.

Kara, E., Ozdilek, H.G. and Kara, E.E., (2004). An Investigation on Physical, Chemical and Bacteriological Quality of Municipally Supplied and Well Water of the Towns and City Centre in the province of Nigde, Turkey. International Journal of Environmental Health Research; 14(2): 151-156.

Karanis, P., (2000). Parasitic Zoonotic Diseases Agents in Drinking Water. Deutsche Tieraeztliche Wochenschrift; 107(8): 311-315.

Karanis, P., Sotiriadou, I., Kartasher, V., Kourenti, C., Tsvetkora, N., and Stojanova, K., (2006). Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. Environmental Research; 102(3): 262-71.

Karmacharya, P.A., Shrestha, R.K., and Shakya, S., (1992). Monitoring of the Kathmandu City Water Supply with reference to Chlorination and Microbiological Quality. Environment and Public Health Organization, Kathmandu, Nepal.

Khadka, M.S., (1994). Water quality of the municipal water supply in Kathmandu Valley. In: water Down Under 94: Groundwater/surface Hydrology common interest Papers: Preprints of Papers: pages: 571-575. Barton, ACT: Institute of Engineers, Australia. National conference publication (Institution of Engineers, Australia: No. 94/10.Availability: http://searh.informit.com.au/documentSummary:dn=752564610435075:res=IELENG ISBN: 085825607X. (Cited 22 Oct 08)

Kravitz, J.O., Nyaphisi, M., Mandel, R., and Peterson, E., (1999). Quantitative Bacterial Examination of Domestic Water Supplies in the Lesotho highlands: Water Quality Sanitation and Village Health. Bulletin of WHO; 77(10):829-833.

Laluraj, C.M., Gopinath, G. and Dineshkumar, P.K., (2006). Ground Water Chemistry of Shallow Aquifers in the Coastal Zones of Cochin, India. Applied Ecology and Environmental Research; 3(1):133-139.

Leuenberger, H., (1983). Trinkwasserqualitat in 13 ausgewahlten Anlagen dos CWSS-Pragrammes in Pokhara Nepal Ausgefuhrt Von H. L in der Praktimumszeit vol II september 19, Dez 1983. NADEL : Nachdiplomastudium for Entwicklungslander.

Lohani, A., (1992). Study in Drinking Water Quality of Koteshwor Area. A dissertation submitted to the Central Department of Zoology in partial fulfillment of the requirements for the award of the degree of Master of Science in zoology, Tribhuvan University, Kirtipur, Nepal.

LSMC, (2002). Lalitpur Sub-Metropolitan City : A Brief Introduction. Lalitpur Sub-Metropolitan City, Pulchowk, Lalitpur, Nepal.

Maharjan, M., (1998). Assessment of Ground Water Quality and Study of Anti-Biotic Resistance and Oligodynamic Action against Some Isolated Enteric Bacteria. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Maharjan, M., and Sharma, A.P., (2000). Bacteriological Quality of Ground Water in Urban Patan and Antibiotic Sensitivity against Isolated Enteric Bacteria. Journal of Nepal Medical Association; 39: 269-274.

Maharjan, M., Joshi, D.D., Joshi, H., (2001). Water Quality Status of Kathmandu Under Aquatox 2000 Program in Nepal. National Zoonosis and Food Hygiene Research Center (NZFHRC), Kathmandu, Nepal.

Miller, Jr.G.T., (2002). Living in the Environment. Principles Connections and Solutions. (12th ed.). Wads Worth Publishing Company, ITF, USA. Pp 476-496.

MoH, (2004). Annual report 2003/04. Government of Nepal, MoH, Department of Health Service, Kathmandu, Nepal.

Momba, M.N.B., and Notshe, T. L., (2003). The Microbiological Quality of Ground Water. Journal of Water Supply Research and Technology- AQUA; 52(1): 67-77.

Mugga, R.G., (1998). Safer Water for Uganda. World heath; 6-7.

Musa, H.A., Shears, P., Elsabag, S. K., (1999). Water Quality and Public Health in Northern Sudan. Journal of Applied Microbiology; 87(5): 676-682.

Nepal Net, (2001). Contamination of drinking water in Nepal. Availability: http://www.panasia.org.sg/nepalnet/water/watercon.html. (Cited on 22 Oct 08)

NESS, (1995). Research on Environmental Pollution and Management. Nepal. Environment & Scientific Services (NESS), Kathmandu, Nepal.

NESS, (1999). Environmental Mapping-Madhyapur Thimi Municipality. Nepal Environment & Scientific Services (NESS). Pp 2-19.

NGO forum, (2006). Traditional Stone Spouts. Enumaration, Mappoing, and Water Quality (Five Municipal Area of Kathmandu Valley 2006). NGO Forum for Urban Water And Sanitation, Kathmandu, Nepal. Pp 6, 9, 19, 23-26, 47.

Park K., (2005). Environment and Health In : Park's Textbook of Preventive and Social Medicine. (18th ed.). M/S Banarsidas Bhanot, Premnagar, India. Pp 519-42.

Paul, M., Wolf, L., Fund, K., Held, I., Winter, J., Elswirth, M., Gallert, C., and Hoeltzl, H., (2004). Microbiological Condition of Urban Ground Water in the Vicinity of Leaky Sewer System. Acta Hydrochimica et Hydrobiologica; 32(4): 351-360.

Pradhan, B., Gruendlinger, R., Fuerhapper, I., Pradhan, P., and Pradhananga, S., (2005). Knowledge of Water Quality and Waterborne Disease in Rural Kathmandu Valley, Nepal. Aquatic Ecosystem Health and Management; 8(3): 277-284.

Pradhan, B., Shrestha, N.P., Sherchan, J.B., and Pradhanang, S., (1995). Water Quality and People's Knowledge About Water Related Diseases : The Case Of Kirtipur Locality, Kathmandu. J.Inst.of Med; 17: 26-31.

Pradhananga, T.M., Thapa, A., Sharma, U., Shahi, R.R., and Shrestha, S., (1993). Water Quality Monitoring of Stone Taps of Pashupati Area. Journal of Nepal Chemical Society; 12: 55-62.

Prasai T., (2002). Drinkig Water Qulaity Assessment of Kathmandu Valley and Antibacterial Property of Medicinal Plants against Enteric Bacteria Isolated From Water. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Prasai T., Lekhak, B., Joshi, D.R., and Baral, M.P., (2007). Microbial Analysis of Drinking Water of Kathmandu Valley. Scientific World; 5(5): 112-114.

Raghu Ram, T.L., (2005). Water Quality Governance and Strategy Option for India. Indian Institute of Management. Pp 4-5.

Rainey, R.C. and Harding, A.K., (2005). Drinking Water Quality and Solar Disinfection. Journal of Water and Health; 3(3): 239-248.

Rawal, S., Shrestha, R.S., Prasai, B.R., Pokharel, B.M., and Takashikubo, (1994).Bacterial Investigation of Drinking Water in Nepal. J. Inst. of Med; 16:120-126.

Regmi, P., (2001). Water Quality Analysis of Stone Taps (Spouts) of Kathmandu City. A dissertation submitted to the Central Department of Microbiology in partial fulfillment of the requirements for the award of the degree of Master of Science in microbiology, Tribhuvan University, Kirtipur, Nepal.

Roberts, L., Chartier, Y., Chartier, O., Malenga, G., Toole, M., and Rodka, H., (2001). Keeping Clean Water Clean in a Malawi Refugee Camp: A Randomized Intervention Trial. Bulletin of WHO; 79(4): 280-285.

Schaffner, M., (2003). Drinking Water Quality Assessment and Improvement in the Jhikhu Khola Catchment, Nepal. Publication Gewaesserkunde, No. 281. Department of Geography, University of Bern, Bern, Germany.

Schraft, H. and Waterworth, L.A., (2005). Enumeration Of Heterotrophs, Faecal Coliforms and *Escherichia Coli* in Water: Comparison Of 3M (TM) Petrifilm (TM) Plates with Standard Plating Procedures. Journal of Microbiological Methods; 60(3): 335-342.

Senhorst, H.A.J., and Zwolsman, J.J.G., (2005). Climate Change and Effects on Water Quality. Water Science and Technology; 51(5): 53-59.

Shakya, R., (2002). Present Drinking Water Quality Status and Control with Sources Supply and Management Monitoring and Drinking Water Management, a Pilot Project in Ward Number 19 and 20 of KMC. National Zoonoses and Food Hygiene Research Centre (NZFHRC), Kathmandu, Nepal.

Sharma, A.P., (1978). The Quality of Drinking Water in Kathmandu, Journal Institutes Sc; 1: 15-22.

Sharma, A.P., (1986). Study of Some Chemicals and Bacteriological Aspects of Drinking Water in Kathmandu. A report submitted to National Committee for Man and Biosphere, Kathmandu, Nepal. Pp 15-22.

Sharma, A.P., (1993). Study of Bacteriological Standard of Drinking Water of Nepal. A report submitted to National Committee for Man and Biosphere, Kathmandu, Nepal.

Sharma, S., (2002). Procuring Water, Foreign Aid and Rural Water Supply in Nepal, Nepal Water Conservation Foundation. (1st ed.). Pp 37-40.

Sharma, S., Bajracharya, R., Sitaula, B.K., and Merg, J., (2005). Water Quality in the Central Himalaya, Asian Institute of Technology. Current Science, Thailand; 89(5): 782.

Sharma, V.K., and Bharadwaj, R.,(2000). Bacteriological Investigation of the Water of Kalayatji Village Pond of Bikaner (Rajasthan). UP Journal of Zoology; 20(1): 79-82.

Sinha, D.K. and Saxena, R., (2006). Statistical Assessment of Underground Drinking Water Contamination and Effect of Monsoon at Hasanpur, J.P. Nagar, India. Journal of Environmental Science and Engineering; 48(3): 157-164.

STIDH, (2004). Records of Waterborne Diseases 2004. Sukraraj Tropical Infectious Disease Hospital, Kathmandu, Nepal.

Tallon, P., Magajna, B., Lofranco, C., and Leung, K.T., (2005). Microbiological Indicators of Faecal Contamination in Water. Water, Air and Soil Pollution; 166(1-4): 139-166.

Thapa, S., (2002). Assessment of Biological Contamination and Water Borne Helminth Parasites in Drinking Water Sources of Ward no 20 KMC. A dissertation submitted to the Central Department of Zoology in partial fulfillment of the requirements for the award of the degree of Master of Science in zoology, Tribhuvan University, Kirtipur, Nepal.

Tiwari, B.N., (1999). Affordability and Willingness to Pay of Water Users in Kathmandu Valley. The Economic Journal of Nepal; 22(2): 53-72.

UNEP, (2001). Nepal : Stae of Environment. United Nations Environment Programs, in collaboration with NOPE/HMGN ; SACEP, ICIMOD and NORAD.

UNICEF, (1987). Children and Women of Nepal, A situation Analysis, UNICEF, Nepal.

Warner, N.R., Levy, J., Harpp, K., and Farruggia, F., (2001). Groundwater Quality in Nepal's Kathmandu Valley: An Assessment of Controlling Site Characteristics. Paper presented at Geological Society of America Annual meeting November 5-8, 2001, Availability: http://gsa.confex.com/gsa/2001AM/finalprogram/abstract_23108.htm. (Cited on 25 June 08).

Warner, N.R., Levy, J., Harpp, K., and Farruggia, F., (2008). Drinking Water Quality in Nepal's Kathmandu Valley: A Survey and Assessment of Selected Controlling Site Characteristics. Hydrology; 16(2):321-334.

Water Aid, (2005). Water Aid 2004/05, Annual Review, Water Aid, Prince Consort House.

WHO, (2007). Health through Safe Drinking and Sanitation. WHO, Geneva.

9. APPENDICES

Table 15. WHO guideline value.

Fc/100 ml*	Grade	Standards	Risk
0	A	Excellent	No risk
0-4	B	Satisfactory	No risk
4-10	C	Suspicious	Low risk
>10	D	Unsatisfactory	High risk
180	E	Polluted	Very high risk

- Fc- Faecal coliform (Source: Shakya, R., 2002)

Table 16. Table Scoreboard MPN for ColiPlate [MR].

No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**
0	**<3**						
1	**3**	25	**76**	49	**182**	73	**388**
2	**5**	26	**79**	50	**188**	74	**403**
3	**8**	27	**83**	51	**194**	75	**418**
4	**11**	28	**87**	52	**200**	76	**434**
5	**13**	29	**90**	53	**206**	77	**451**
6	**16**	30	**94**	54	**213**	78	**469**
7	**19**	31	**98**	55	**219**	79	**489**
8	**22**	32	**102**	56	**226**	80	**510**
9	**25**	33	**106**	57	**233**	81	**534**
10	**28**	34	**110**	58	**240**	82	**559**
11	**30**	35	**114**	59	**247**	83	**587**
12	**33**	36	**119**	60	**255**	84	**619**
13	**36**	37	**123**	61	**263**	85	**654**
14	**39**	38	**127**	62	**271**	86	**694**
15	**43**	39	**132**	63	**280**	87	**740**
16	**46**	40	**136**	64	**289**	88	**794**
17	**49**	41	**141**	65	**298**	89	**858**
18	**52**	42	**146**	66	**307**	90	**938**
19	**55**	43	**151**	67	**317**	91	**1038**
20	**59**	44	**156**	68	**328**	92	**1174**
21	**62**	45	**161**	69	**339**	93	**1370**
22	**65**	46	**166**	70	**350**	94	**1696**
23	**69**	47	**171**	71	**362**	95	**2424**
24	**72**	48	**177**	72	**375**	96	**> 2424**

Table 17. Scoreboard MPN for ColiStripMR.

No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**	No. of wells with positive reaction	**MPN / 100 ml sample**
0	**<16**						
1	**16**	5	**94**	9	**213**	13	**469**
2	**33**	6	**119**	10	**255**	14	**619**
3	**52**	7	**146**	11	**307**	15	**938**
4	**72**	8	**177**	12	**375**	16	**> 938**

Table 18. Guideline Values for Bacteriological quality.

Organism	Unit	Guideline value	Remark
A. Piped water supplies			
A.1 Treated water entering the distribution system			
Faecal Coliforms	Number/100 ml	0	Turbidity <1 NTU; for disinfection with chlorine, pH preferably <8.0 free chlorine residual 0.2-0.5 mg/litre following 30 minutes minimum contact.
A.2 Untreated water entering the distribution system			
Faecal coliforms	Number/100ml	0	In 98% samples examined throughout the year in the case of large supplies when sufficient samples are examined.
Coliform Organisms	Number/100ml	0	
Colifrom Organisms	Number/100ml	3	In an occasional sample, but not in consecutive samples
A.3 Water in distribution system			
Faecal coliforms	Number/100ml	0	In 95% samples examined throughout the year in the case of large supplies when sufficient samples are examined
Colifrom organisms	Number/100ml	0	
Colifrom organisms	Number/100ml	3	In an occasional sample, but not in consecutive samples
B. Unpiped water supplied			
Faecal coliforms	Number/100ml	0	
Coliform organisms	Number/100ml	10	Should not occur repeatedly, If occurrence is frequent and if sanitary protection cannot be improved an alternative source must be found if possible.
C. Bottled drinking water			
Faecal coliforms	Number/100ml	0	Source should be free from faecal contamination
Coliform organisms	Number/100ml	0	
D. Emmergency water supplies			
Faecal coliforms	Number/100ml	0	Advise public to boil water in case of failure to meet WHO guideline values
Coliform organisms	Number/100ml	0	

Table 19. Inventory of water samples taken in the study*.

S.N.	Sources	Status	Location	S.N.	Sources	Status	Location
1	Well	public	Lagankhel	41	Well	Public	Kusunti
2	Well	public	Mahalaxmithan	42	Well	Public	Mathilokusunti
3	Well	public	Mahalaxmithan	43	Well	Personal	Kusunti
4	Well	public	Kumaripati	44	Well	Personal	Kusunti
5	Well	public	Thasikhel	45	Well	Personal	Manimandal
6	Well	public	Thasikhel	46	Well	Personal	Mathilokusunti
7	Well	Personal	Kumaripati	47	Well	Personal	Ekantakuna
8	Well	Personal	Lagankhel	48	Well	Personal	Ekantakuna
9	Well	Personal	Thasikhel	49	Well	Personal	Pulchowk
10	Well	Personal	Kumaripati	50	Well	Personal	Pulchowk
11	Well	Personal	AVM chowk	51	Well	Personal	Pulchowk
12	Well	Personal	AVM chowk	52	Well	Personal	Nakkhu
13	Well	Personal	Jawalakhel	53	Well	Personal	Nakkhu
14	Well	Personal	Jawalakhel	54	Well	Personal	Bagdole
15	Well	Personal	Kumaripati	55	Well	Personal	Bagdole
16	NWSC tap	Public	Mahalaxmithan	56	NWSC tap	Public	Kusunti
17	NWSC tap	Public	AVM chowk	57	NWSC tap	Public	Bagdole
18	NWSC tap	Personal	Lagankhel	58	NWSC tap	Personal	Manimandal
19	NWSC tap	Personal	Lagankhel	59	NWSC tap	Personal	Mathilokusunti
20	NWSC tap	Personal	Thasikhel	60	NWSC tap	Personal	Mathilokusunti
21	NWSC tap	Personal	Thasikhel	61	NWSC tap	Personal	Ekantakuna
22	NWSC tap	Personal	Thasikhel	62	NWSC tap	Personal	Pulchowk
23	NWSC tap	Personal	AVM chowk	63	NWSC tap	Personal	Pulchowk
24	NWSC tap	Personal	Jawalakhel	64	NWSC tap	Personal	Pulchowk
25	NWSC tap	Personal	Jawalakhel	65	NWSC tap	Personal	Nakkhu
26	NWSC tap	Personal	Kumaripati	66	NWSC tap	Personal	Nakkhu
27	NWSC tap	Personal	Kumaripati	67	NWSC tap	Personal	Bagdole
28	Stored water	Personal	Lagankhel	68	Stored water	Personal	Kusunti
29	Stored water	Personal	Lagankhel	69	Stored water	Personal	Kusunti
30	Stored water	Personal	Lagankhel	70	Stored water	Personal	Kusunti
31	Stored water	Personal	Mahalaxmithan	71	Stored water	Personal	Manimandal
32	Stored water	Personal	Mahalaxmithan	72	Stored water	Personal	Manimandal
33	Stored water	Personal	Mahalaxmithan	73	Stored water	Personal	Manimandal
34	Stored water	Personal	AVM chowk	74	Stored water	Personal	Ekantakuna
35	Stored water	Personal	AVM chowk	75	Stored water	Personal	Ekantakuna
36	Stored water	Personal	Jawalakhel	76	Stored water	Personal	Pulchowk
37	Stored water	Personal	Jawalakhel	77	Stored water	Personal	Pulchowk
38	Stored water	Personal	Kumaripati	78	Stored water	Personal	Pulchowk
39	Stored water	Personal	Kumaripati	79	Stored water	Personal	Pulchowk
40	Stored water	Personal	Kumaripati	80	Stored water	Personal	Bagdole

Table 19 contd……..

S.N.	Sources	Status	Location	S.N.	Sources	Status	Location
81	Well	public	Mikhabahal	121	Well	Public	Talache
82	Well	public	Mikhabahal	122	Well	Public	Talache
83	Well	public	Mikhabahal	123	Well	Public	Chobunani
84	Well	public	Mikhabahal	124	Well	Personal	Chakupat
85	Well	public	Na:tole	125	Well	Personal	Chakupat
86	Well	public	Chhayabahal	126	Well	Personal	Imukhel
87	Well	public	Chhayabahal	127	Well	Personal	Imukhel
88	Well	public	Gabahal	128	Well	Personal	Washahiti
89	Well	public	Shreebahal	129	Well	Personal	Washahiti
90	Well	Personal	Chhayabahal	130	Well	Personal	Shankamul
91	Well	Personal	Chhayabahal	131	Well	Personal	Chobunani
92	Well	Personal	Phimbahal	132	Well	Personal	Chobunani
93	NWSC tap	public	Kuchenani	133	NWSC tap	public	Banglamukhi
94	NWSC tap	public	Phimbahal	134	NWSC tap	public	Banglamukhi
95	NWSC tap	public	Chhayabahal	135	NWSC tap	Personal	Washahiti
96	NWSC tap	Personal	Chhayabahal	136	NWSC tap	Personal	Washahiti
97	NWSC tap	Personal	Chhayabahal	137	NWSC tap	Personal	Shankamul
98	NWSC tap	Personal	Shreebahal	138	NWSC tap	Personal	Shankamul
99	NWSC tap	Personal	Phimbahal	139	NWSC tap	Personal	Ekache
100	NWSC tap	Personal	Phimbahal	140	NWSC tap	Personal	Chobunani
101	NWSC tap	Personal	Phimbahal	141	NWSC tap	Personal	Chobunani
102	Stone tap	Public	Na:tole	142	Stone tap	public	Imukhel
103	Stone tap	Public	Na:tole	143	Stone tap	public	Imukhel
104	Stone tap	Public	Na:tole	144	Stone tap	public	Washahiti
105	Stone tap	Public	Na:tole	145	Stone tap	public	Washahiti
106	Stone tap	Public	Chhayabahal	146	Stone tap	public	Banglamukhi
107	Stone tap	Public	Chhayabahal	147	Stone tap	public	Banglamukhi
108	Stone tap	Public	Chhayabahal	148	Stone tap	public	Banglamukhi
109	Stone tap	Public	Chhayabahal	149	Stone tap	public	Banglamukhi
110	Stored water	Personal	Chhayabahal	150	Stored water	Personal	Chakupat
111	Stored water	Personal	Mikhabahal	151	Stored water	Personal	Chakupat
112	Stored water	Personal	Mikhabahal	152	Stored water	Personal	Chakupat
113	Stored water	Personal	Shreebahal	153	Stored water	Personal	Imukhel
114	Stored water	Personal	Shreebahal	154	Stored water	Personal	Imukhel
115	Stored water	Personal	Shreebahal	155	Stored water	Personal	Banglamukhi
116	Stored water	Personal	Gabahal	156	Stored water	Personal	Talache
117	Stored water	Personal	Gabahal	157	Stored water	Personal	Talache
118	Stored water	Personal	Gabahal	158	Stored water	Personal	Shankamul
119	Stored water	Personal	Kuchenani	159	Stored water	Personal	Ekache
120	Stored water	Personal	Na:tole	160	Stored water	Personal	Ekache

- S.N.= sample number, (S.N. 1-40 ward number 5, 41-80 ward number 13, 81-120 ward number 21, and 141-160 ward number 22)

Table 20. Field Data Sheet.

Community ----------

Date	Sample No.	Sampling Time	Source	Type of Test	Sample Volume	Comments

Table 21. Conformation of Results with Coliplate™.

Date	Sample No.	Source	Coliplate™ Results							
			Positive Results of H_2S		Coliforms			*E. coli*		
			Volume	#days	# + wells	# - wells	MPN/100 ml	# + wells	# - wells	MPN/100 ml

Table 22. Data collection sheets-H_2S test 10 ml samples volumes.

Date	Sample No.	Source	H_2S 10 ml volume					Notes
			Day 1	Day 2	Day 3	Day 4	Day 5	Comments

Water borne diseases caused by protozoan, helminthes, and bacteria.

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Protozoal infections			
Amoebiasis (hand-to-mouth)	Protozoan (*Entamoeba histolytic*) (Cyst-like appearance)	Sewage, non-treated drinking water, flies in water supply	Abdominal discomfort, fatigue, weight loss, diarrhoea, gas pains Fever, abdominal pain
Cryptosporidiosis (oral)	Protozoan (*Cryptosporidium parvum*)	Collects on water filters and membranes that cannot be disinfected, animal manure, seasonal runoff of water.	Flu-like symptoms, watery diarrhoea, loss of appetite, substantial loss of weight, bloating, increased gas, stomach
Cyclosporiasis	Protozoan parasite (*Cyclospora cayetanensis*)	Sewage, non-treated drinking water	cramps, nausea, vomiting, muscle aches, low-grade fever, and fatigue
Giardiasis (oral-fecal) (hand-to-mouth)	Protozoan (*Giardia lamblia*) Most common intestinal parasite	Untreated water, poor disinfection, pipe breaks, leaks, groundwater contamination, campgrounds where humans and wildlife use same source of water. Beavers and muskrats act as a reservoir for *Giardia*.	Diarrhoea, abdominal discomfort, bloating, gas and gas pains
Microsporidiosis	Protozoan (*Microsporidia*), but closely related to fungi	The genera of *Encephalitozoon intestinalis* has been detected in groundwater, swimming pool via AIDS patients and the origin of drinking water	

…… Contd

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Parasitic Infections			
Schistosomiasis (immersion)	*Schistosoma*	Contaminated fresh water with certain types of snails that carry schistosomes	Rash or itchy skin. Fever, chills, cough, and muscle aches
Dracunculiasis	*Dracanculus medinensis*	drinking water containing infective cyclops	Allergic reaction,urticaria rash, nausea, vomiting, diarrhea, asthmatic attack.
Taeniasis solium	*Taenia solium*	contaminate drinking water with eggs	intestinal disturbances, neurologic manifestations, loss of weight, cysticercosis
Fasciolopsis	*Fasciola*	contaminated drinking water with encysted metacercaria	GIT disturbance, diarrhea, liver enlargement, cholangitis, cholecystitis, obstructive jaundice.
Hymenolepiasis nana	*Hymenolepis nana*	contaminated drinking water with eggs	mild GIT symptoms, nervous manifestation
Hyatidosis	*Echinococcus granulosus*	contaminated drinking water with eggs	hyatid cyst press on bile duct and blood vessels, if it ruptured cause anaphylactic shock.
Coenurosis	*Multiceps multiceps*	contaminated drinking water with eggs	increases intacranial tension

……Contd

Disease and Transmission	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Ascariasis	*Ascaris lumbricoides*	Contaminated drinking water with eggs	Loefflers syndrome in lung, nausea, vomiting, diarrhoea, malnutrition, underdevelopment,
Enterobiasis	*Entrobius vermicularis*	Contaminated drinking water with eggs	peri-anal itch, nervous irritability, hyperactivity and insomnia

Bacterial infections

- Botulism - *Clostridium botulinum* bacteria - gastro-intestinal food/water borne; can grow in food
- Campylobacteriosis
- Cholera - *Vibrio cholerae* bacteria - gastro-intestinal often waterborne
- Chronic granulomatous disease - caused by the Mycobacterium marinum infection and localized in skin, frequently occurred with aquarium keepers.
- Diarrheal disease due to *E. coli*.
- Dysentery - *Shigella/Salmonella* bacteria - gastro-intestinal food/water
- Legionellosis - cause Pontiac fever and Legionnaires' disease
- Leptospirosis-
- Typhoid - *Salmonella typhi* bacteria - gastro-intestinal water/food borne. Salmonellosis - due to many *Salmonella* species. Water/food/direct contact borne.
- Vibrio illness caused by the bacteria of *Vibrio vulnificus*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* commonly found in seafood and recreational water.