A STUDY ON MICROBIOLOGICAL AND CHEMICAL QUALITY OF WATER OF KATHMANDU

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BY
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RECOMMENDATION

This is to certify that Mrs. Rama Gyewali has completed her dissertation work entitled "**A study on Microbiological and Chemical quality of water of Kathmandu**" as a partial fulfillment of the M.Sc. degree in Microbiology under our supervision. To our knowledge her work has not been submitted for any other degree.

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ABSTRACT

The study assessed the water quality of Kathmandu, water samples were taken from seven different sources for bacteriological investigation and analyzed for their Bacterial count and coliform count. Water samples were collected from various sources such as river water, tank water, tap water etc.

In this study, all the water samples showed the growth of coliform bacteria and *Salmonella species*. The highest bacterial count was found to be $6.4X10^6$ CFU/ml in sample S_6 (River water sample) and lowest bacterial count was found to be $3.0X10^3$ CFU/ml in sample S_5 (Kuleshwor tap water). Similarly, the highest coliform count was 1100 CFU/ml in sample S_6 and lowest coliform count was found to be 500 CFU/ml in sample S_3 (Sundharighat Tank).

Chemical analysis shows that the pH of all the collected samples was found to be within the limit of WHO guidelines (7.0-8.5). Other parameters like total hardness, BOD and Sulphates concentrations were found to be below the WHO guidelines where as chloride contents exceeds the limit in five samples.

The result and analysis of this study on chemical and microbiological quality of samples from different water source shows that coliforms were present. Hence, all the water samples were microbiologically unsafe for drinking and need treatment.

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ABBREVIATION

ADB	-	Asian Development Bank
APHA	-	American Public Health Association
CBS	-	Central Bureau of Statistics
CEDA	-	Centre for Economic Development and Administration
ISVI	-	Italian International Co-operation
DWSS	-	Department of water supply and sewerage
ENPHO	-	Environment and Public Health Organization
FAO	-	Food and Agricultural Organization
GTZ	-	German Technical Co-operation
HMG	-	His Majesty's Government
ICIMOD	-	International Centre for Integrated Mountain Development
IUCN	-	International Union for Conservation of Nature
IWTC	-	International Women's Tribune Centre
KCN	-	Potassium Cyanide
MLD	-	Ministry of Local Development
RONAST	-	Royal Nepal Academy of Science and Technology
UNEP	-	United Nations Environment Protection
WHO	-	World Health Organization
WSSB	-	Water Supply and Sewerage Board

Chapter-I

1. INTRODUCTION

Water is main source for living organism and is most precious gift of nature to man kind and vital on the earth, without water there is no possibility of life. Water is a part of human environment and it can also be the carrier of pathogenic microbes. Water is the basic life supporting resource and a very important factor in the social, cultural, economic and ecological development of a country and human civilization.

Water covers about three-quarters of the Earth's surface however 97.4% is salt water in Oceans and 1.8% is frozen in the Polar Regions, fresh water constitutes only 0.8% of the world's supply and nobody knows just what portion of this amount is contaminated. Only 30% of the world's people have a guaranteed supply of treated water. The Remaining 70% depends on wells, bore holes and other uncertain sources of water supply, all liable to contamination. About 80 percent of all diseases in the developing world are related to unsafe water supply and inadequate sanitation. Due to the absence of an adequate, reliable water supply and sanitation services, more than 15 million children aged 0 to 4 die each year. It has been estimated that today, 100 million more people have to drink dirty water than in 1975 and 400 million more than five years ago have no sanitation.

Nepal is a mountain kingdom endowed with abundant water resources but the majority of Population do not have access to clean drinking water. Nepal faces enormous challenge to provide potable drinking water to all its population. The Population of Nepal is Predominantly Rural with only about 10% living in urban area. At present, the population having access to piped water facility is estimated to be 47 percent (WHO, 1994)

Water is the scarce but a previous commodity on the earth. It is getting scarer everyday as communities, industries and agriculturalist pour their muck and untreated wastes into the nearest sink. Its demand for multiple uses such as for drinking, cleaning, cooking, electricity, industry, irrigation, transport, fishery, development projects of resource conservation etc.

In Nepal, cities don't have proper water supply system. Drinking water of must communities municipalities is obtained from surface source-river, stream, lakes and ponds etc. Such natural water supplies particulars stream and river are likely to be polluted with domestic and industrial waste. Sewage may contain millions of bacteria per milliliter including the coliform, anaerobic spore forming bacilli streptococci the protect group and other type originating in the intestinal tract of human. Sewage also potential source pathogenic protozoa and viruses. The causative agents of dysentery, cholera and typhoid fever may occur in sewage. There are frequent reports of the spreads of typhoid, diarrhea and cholera through water supply in Nepal and far communities which devoid of water supply face these problems frequently (MOH/WHO, 1994).

Water Pollution is the most serious environmental quality issue all over the world, yet the people is less aware and gives little emphasis on the vital connection variety of pathogenic micro-organisms and water has been found to be vehicle for the transmission of communicable diseases. Several types of pathogenic micro-organisms are found in polluter water which leads to health hazards-many studies have implicated that the poor water is a principle factor for morality and morbidity associated with enteric diseases. Thus water may be extremely dangerous when it becomes the vehicle of transmission of disease, it causes exclusive outbreaks. Basically, water borne illness are associated with ingestion of water containing pathogenic microorganisms is bacteria, protozoa and viruses which is responsible for must mortality and morbidity in developing countries. (WHO, 1993)

In Nepal rural People drink water from Rivers, Ponds, Canals, Springs, aquifers, well, tube wells and stone spouts which are polluted and sometimes quiet far from villages.

In the hills of Nepal, woman and girls must walk for several hours up and down steep paths to collect water from invariably locally polluted sources, many people collect water from invariably locally polluted sources. Many people collect water from sources away from the point of user store water in sanitary conditions in the house hold even water sources are free from contamination , house hold, storage , pots, tanks and insanitation around household may be maintained (CBS,1995)

Man's views on water pollution are also best on aesthetic values, appearance, taste and colour of drinking water is matter of high priority. In Rural communities, analysis of all chemical parameters and bacteriological parameters is impossible and far of thought. Rural people are unknown about these factor and problems created by them not only in rural area but also in urban area of Nepal, there is not facility to test all these parameters and these test are not often done. Rural Consumers have no means of judging the safety of their drinking water themselves. The provision of drinking water that is not only safe but also pleasing in appearance, test and odour is a matter of high priority. there are a number of water constituent that are of no direct consequence to health at the concentration at which they normally occur in water but which never the less may be objectionable to consumer various reasons(WHO, 1993).

It is well documented in the UNICEF situation Analysis (Unicef, 1987), in Nepal water and hygienic related disease are responsible 8% of all death in general population. According to the country health profile (MOH/WHO, 1994), it shows that infective and parasitic disease constitutes the largest single

(31.27%) of morbidity in the general population. Diarrhea diseases, arising mainly from unhygienic drinking water and sanitary conditions of the water environment account for nearly 1/3rd of child deaths. WHO reported that diarrhea is the second biggest killer and total mortality all over the world, (WHO, 1995). A survey of hospital record showed high incidence (36%) of the water borne diseases. Amoebic dysentery is the most universally prevalent water borne disease through out the country and other very common diseases are dysentery, gastroenteritis, typhoid fever, giardiasis and worms infestation prevalent rate of diarrhea is about 15.9% for all age and 40% among children under five. Approximately 44,000 children under five die of diarrhea each year. Typhoid fever reported attack rate is 30 per thousand population (MOH/WHO, 1994).

In many developing countries, drinking water quality often remains unevaluated because of the high cost of specialized water quality testing. Similar to other developing countries Nepal is in the preliminary stages of water supply development and treatment. The Country could employ and highly benefit from those water supply and treatment Methodologies which involve less capital expenditure operation and maintenance. The Maximum utilization of local resources in construction and operation and minimum utilization of imported materials and equipment will be imported to eliminate operational maintenance problems. The Study was concentrated on present status of water quality of Kathmandu. It also aims to find out the pathogenic micro organisms in river water and drinking water.

CHAPTER-II

2. OBJECTIVES

2.1 General Objectives

To assess chemical and microbiological quality of river water and drinking water of Kathmandu.

2.2 SPECIFIC OBJECTIVES

1. To study some of the chemical parameters of water.
2. To study the faecal indicator and pathogenic bacteria in natural sources.
3. To estimate total coliform and detect *Salmonella* sps from water.

Chapter-III

3. LITERATURE REVIEW

3.1 WATER POLLUTION

Water pollution began with the foundation of the first cities, seven thousand years ago. In history of water pollution, it was generally understood is a local problems without significant effect on the regional or continental scale. However the extent of water pollution has progressed from a local scale to regional continental and now global contamination of water arising from long-range atmospheric transport was found in Antarctic ice. (Meybeck *et. al.* 1990).

The water pollution is accessed on the basis of certain parameters.

1. Physical used to ascertain Temperature, Turbidity, Conductivity, Colour, Suspended, Dissolved and Total solids.
2. Chemical used to ascertain inorganic matter such as acidity, alkalinity, Salinity including several insoluble inorganic materials, soluble salts of organic matters.
3. Physiological used to ascertain taste and odour.
4. Biological used to ascertain bacteria of coliforms.

Portability of water can be determined only by chemical and bacteriological, Laboratory tastes. Chemical analysis indicates whether water is polluted and provides other useful information however it is not sensitive or specific enough to detect minor degree of sewage contamination. Bacteriological tests have been designed which are extremely sensitive and specific in revealing evidence of pollution (Michael *et. al.*, 1979.)

3.1.1 Indicator of Water Pollution

3.1.1.1 Microbial Indicators of Water Quality

The Detection of all the disease causing organism is not possible for the high cost involving and time consuming techniques. It is therefore normal practice to detect and enumerate organism present in large number in human and warm blooded animal intestine and faeces as indicators of bacteriological pollution. Examination for faecal indicator organism is the most sensitive of specific way of assessing the hygienic quality of water. Faecal indicators should be universally present in high numbers in the faeces of humans and warm blooded animals and readily detectable by simple methods. The maior indicator organism of faecal pollution *Escherichia coli*, the thermo tolerant and other coliform bacteria, the faecal streptococci and spores of sulfite reducing clostridia (WHO,1993).

a) *Escherichia coli*

Escherichia coli is a member of the family Enterobactericeae and is characterized by possession of the enzymes β-glactosidase and β-glucononidase. The Enterbacteriaceae is are gram negative bacilli that are either motile with peritrichus flagella or non motile, grow both anaerobically and an aerobically on simple laboratory media and on MacConkey bile salt Lactose agar, are oxidase negative , catalase positive and reduce nitrates to nitrites. They ferment glucose in peptone water with the production of either or acid and gas. The characters of *Escherichia coli* that distinguish it from other enterobacteria are that it motile forms, gas from glucose, ferments lactose, produce indole, gives a positive methyl red reaction and a negative Voges-Prokauser reaction and does not utilize citrate, grow in KCN, liquefy gelatin.

Escherichia coli is abundant in human or animal faeces, and it is found in sewage, freaked effluents and all natural waters and soil that are subject to

recent faecal contamination. It is the commonest cause of urinary tract infection (UTI) and is commonly present in appendix abscess, peritonitis, cholecystitis, Septic wounds and bedsores. They may infect the lower respiratory passages or cause bacteria and endotoxic shock of gastroenteritis (Diarrhea). It grows at 44-45^{o}c on complex media, lactose fermenter and mannitol fermenter with production of acid and gas. *E.coli* is abundant in human and animal faeces where it may attain concentrations in fresh faces of 10^{9} per gram (WHO, 1993).

b) Coliform Bacteria

Coli form organisms have long being recognized of a suitable microbial indicator of drinking water quality, largely because they are easy to detect and enumerate in water. The term coli from are easy to detect and enumerate in water. The term “Coliform organism's" refers to gram negative rod shaped bacteria capable of growth in the presence of bile salts and able to ferment lactose at 35-37°c with the production of acid, gas and aldehyde within 24 to 48 hours. They are also Oxidise negative and non-spore forming coliform group includes genus, *Escherichia, Klebsella, Enterobacter* and *Citrobacter*. The levels of *coliform* organisms present in the drinking water should not exceed the maximum permissible value of less than one cell per 100 ml of water (WHO, 1993).

c) Fecal Streptococci

The term “fecal Streptococci" refers to those streptococci generally present in the faeces of humans and animals. All Posses the Lancefield group D antigen. They belong to the general enterococcus and streptococcus. The genus streptococus consists of chain forming Gram positive cocci that are facultative anaerobes streptococci are catalase negative and mainly separated on the type of haemolysis produced on Blood agar media. Group D streptococci includes *S.faecalis, S.faecium, S.lurans, S.qvium, S.bovis. S.equinus.* These may cause uninary tract infection, endocarditic, bilary tract infection, suppurative

abdominal lesions and ear infections in man. Three species streptococcus *S.faecalis , S.faecium, S.durans* are commonly referred to as " *enterococci"* or *faecal streptococci*(Cheesbrough,1984).

The Primary Value of faecal streptococci in water quality examination is therefore as additional indicators of treatment efficiency.

d) *Salmonella sps*.

The genus *salmonella* is also a member of Enterobateriaceae but not a colifrom group member. It is frequently present in water from different sources like faeces, urine, soils etc. thus regularly tested in water quality tests. It is fermentative facultatively anaerobic, oxidase, negative. It is gram negative generally motile, small rods, non-Lactose fermenting, urease negative, citrate utilizing acetyl methyl carbinol negative and KCN negative. It is non-capsulate and non-sporing bacilli and species of salmonella that invade the blood and cause severe salmonellosis and typhoid fever. A Typhoid fever and salmonellosis are major cause of illness and death in developing countries.

e) Clostridia and other Alternative Indicators

Sulphite reducing clostridia are anaerobic , spore forming organisms of which clostridium perfringens is normally present in faeces of warm blooded animals but in much smaller number than *E.coli* , clostridial spores can survive in water much longer than organisms of the coliform group and will resist disinfection. Thus they have a special value but are not routinely used for monitoring of distribution systems.

Besides, routinely tested indicators, there are other important organisms which are found in water sources and causes harmful diseases. Vibrio cholera is the cause of cholera epidemics attributed to spread by ingesting faecal contaminated water. Besides these, the Bacteriophage have been proposed as

indicators of water quality because of their similarly to human enteroviruses and their easy detection in water.

3.1.1.2 Physico-Chemical Parameters of Water

However, chemical analysis is not as sensitive as microbial analysis but it can provide useful information's and cannot be neglected some of the important parameters under study were Dissolved oxygen(DO), PH, Nitrates, Nitrites, Phosphates, Sulphates and Chlorides etc. (APHA, 1995).

a) Temperature

Temperature is one of the important parameter of water and is basically important for its effects on the chemistry and biological reactions in the organisms in the water. It is important in the determination of various other parameters such as PH, Conductivity, Saturation level of gases and alkalinity etc. Organisms in water have varying sensitivities to temperature. The organisms with high resistance to temperature fluctuations are called "Eruthothermic" while the organisms with low tolerance are referred as "Stenothermic".

b) pH

pH is defined as the negative $\log_{10}$ of hydrogen ion concentration. It is the major of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. No health based guideline values are proposed for pH. Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters careful attention to pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection. Although pH has no direct adverse effects on health, however a lower value below 4 will produce sour taste and higher value above 8.5, an alkaline taste. The pH value of drinking water from any sources should be within range 6.5 to 8.5.

c) Hardness

Hardness in water is caused by dissolved calcium and magnesium. It is usually expressed as the equivalent quantity of calcium carbonate. Hardness is the property of water which prevents the lather formation with soap and increase the boiling point of waters. Hardness has no known adverse effects on health; however some evidence has been given significant inverse relationship between hardness of drinking water and cardiovascular diseases. No health based guideline in water may affect its acceptability to the consumer in terms of taste and scale deposition.

d) Dissolved Oxygen (DO)

Dissolved oxygen is one of the most important parameters in water quality assessment and reflects the physical and biological processes prevailing in the waters. The dissolved oxygen content of water is influenced by the raw water temperature, Composition, treatment and any chemical or biological processes taking place in the distribution system. Depletion of dissolved oxygen in water supplies can encourage the microbial reduction of nitrate to nitrate and sulfate to sulfide giving rise to odour problems. It can also cause an increase in the concentration of ferrous iron solution.

Oxygen can be rapidly removed from the waters by discharge of the oxygen demanding wastes. Inorganic reluctants like H_2S, NH_3 and Nitrites tend to decrease dissolved oxygen in water. Low Contamination by organic matter. Presence of dissolved oxygen is essential to maintain the higher forms of biological life in the water. No health based guideline value has been recommended for dissolved oxygen. Higher the DO Value, better the water quality.

e) Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) is the amount of oxygen utilized by micro-organisms stabilizing the organic matter. The demand for oxygen is proportional to the amount of organic waste to be degraded aerobically. BOD gives a qualitative index of the organic substances which are degraded quickly in short period of time.

The principle of the method involves, measuring the difference of the oxygen concentration between the sample and after incubating for 5 days at 20°c. Higher the BOD value indicates poor water quality. Types of micro organisms, PH Presence of toxins, some reduced mineral matter and nitrification process are the important factors influencing BOD test.

f) Nitrites and Nitrates

Nitrites and Nitrates are naturally occurring ions that are part of the nitrogen cycle. Naturally occurring nitrate levels in surface and ground water are generally a few milligrams per liter high amount of nitrates are generally indicative of pollution.

Nitrite is a very unstable ion and gets converted into either ammonia or nitrate depending upon the conditions prevailing in the water. Presence of even a small quantity of nitrite will indicate the organic pollution and availability of partially oxidized nitrogenous matter. The high concentration of nitrates and nitrites can also cause "Blue Baby" disease (Methanoglobinemia) in infants.

g) Phosphates

Phosphorus in the natural fresh waters is present mostly in inorganic form as $H_2PO_4^-$, HPO_4^{-2}, and PO_4^{-3}. Phosphorus being important constituents of biological system may also be present in the organic form. The major sources of Phosphorus are domestic, sewage, detergents, agricultural effluents with

fertilizers and industrial waste waters. The higher concentration of phosphorus is therefore is indicative of pollution.

h) Sulphate

Sulphate is an important constituent of hardness with calcium and magnesium. It is naturally occurring anion in all kinds of natural waters. Biological oxidation of reduced sulphur to sulphates also increases its concentration. Discharge of industrial waste and domestic sewage in waters tend to increase its concentration. Sulphate is one of the least toxic anions, however, catharsis, dehydration and gastrointestinal imitation have been observed at high concentrations. The presence of sulphate in drinking water may also cause noticeable taste and may contribute to the corrosion of distribution systems. No health based guideline values have been derived for sulphate.

i) Chlorides and Residual chlorine

Chloride in drinking water originates from natural sources, sewage and industrial effluents. No health-based guideline value is proposed for chloride in drinking water. However, chloride concentrations in excess of about 250 mg/ltr. can give rise to detectable taste in water.

Residual chlorine in drinking water after disinfection with chlorinated disinfectant should be 0.2-0.5 mg/ltr. Free chlorine beyond the limit 0.2-0.5 mg/ltr. can have deleterious effects on consumers. In water chlorine reacts to form hypochlorous acid and hypochlorites. The guideline value of free chlorine in drinking water is based on a TDI (Tolerable Daily Intake).

3.1.1.3 Microbial Examination of Drinking Water in Nepal

Most water pollution studies have examined the quality of drinking water supplied to the Kathmandu valley, particularly to its urban areas. Very few places have been survived elsewhere in the country. However, the extent of drinking water contamination in the areas covered by pollution studies suggests that the problem is nation wide.

Sharma (1978) studied the quality of drinking water supplied to the household of the Kathmandu valley. He performed *coliform* tests on water samples from 39 localities. He found that the number of *coliform* cells per 100ml of water ranged from 4 to 460.

DISVI (1989) came out a more detailed and comprehensive study to assess the quality of Kathmandu valley drinking water supply and identify weaknesses in the city's public water system. The quality of drinking water was monitored for a period of six months, from January to June. Drinking water samples were collected from 58 sampling points, 7 water treatment plants and reservoirs, 7 hospitals storage tanks, and 44 water taps, a total of 472 samples were tested.

CEDA (1989) tested water samples from different localities in Kathmandu. CEDA study found that all samples were contaminated with faecal materials. None of the tap and ground water sources were safe for drinking.

DISVI (1990 b) carried out a bacteriological tests of drinking water quality assessment in seven rural areas of Ilam in Eastern Nepal. The seven villages were surveyed and samples were collected from 36 households and water sources including springs, spring well, aquifers (kuwa), rivers and river water reservoir. Bacteriological, physical and chemical parameters were tested. Study

found that unacceptable levels of faecal *coliform* bacteria ranges from 2 to 2,400 cells per 100ml.

Joshi (1987) carried out bacteriological tests of drinking water sources of two villages central Nepal nearer to the capital: chaubas (shivpuri) and syabru (Langtang). The coli form count ranged from 5-100 cells per 100ml of water. In chaubas, water from uncovered springs showed contamination within the range of 20-100 cells/ml.

CEDA (1989) reported that 5 percent of Kathmandu population regularly used water form dug wells and stone spouts. Sharma (1986) studies the water quality from their sources and reported coliform bacteria counts from 0 to 460 per 100ml. during summer.

ENPHO/DISVI (1990) investigated bacteriological quality of ground water in Kathmandu. Samples were collected from stone spouts in 21 localities. The lowest average faecal coliform densities were observed in the samples from Bhatbhateni (8 cells/100ml), Balajutar (1 cell/100ml) and Sundhara (19 cells/100ml). The highest densities were observed in the samples from Bhimsen than (37,602 cells/100ml) and Narayanhity contained on average density of more than 100 cells/100ml.

DISVI (1990c) accessed ground water quality in seven rural areas of Morang, Sunsari, Jhapa, Siraha and Saptari in the Eastern Development region of Nepal. A total of 164 samples were tested. For chemical and Bactriological properties, 70 from iron removal plants of the project, 20 from non-project tubewell, 12 from non project dug wells and 39 from households. The bacteriological quality of water from tubewells well found to be far better than that of water from dugwells. About 90 percent of the samples from project tubewells and

less than 10 faecal *coliform* cells per 100ml, 8 percent have 10-100 cells/100ml and only 2 percent had more than 100 cells/100ml.

Sharma (1993) carried out microbiological examination of water samples from different major cities of Nepal. He found that the highest coli form count was 2400 cells per 100 ml of water in Kathmandu, 4800 cells per 100 ml in Pokhara. He isolated enteric pathogenic bacteria such as *Salmonella typhi*, *Proteus* sps, *Klebsiella sps* and *Shigella sps* from the water samples.

Sharma (1986) in a same follow-up study examined water samples from different sources from Kathmandu and Lalitpur area. He found contamination level reached 4800 coliform cells per 100 ml. Water samples were taken in the dry summer season, the rainy season and winter season. The coliform bacteria count ranged from 0 to 4800 during the rainy season, 0 to 75 in winter and 0 to 460 per 100 ml in the summer months.

3.1.1.4 Status of Water Supplies in Nepal

The history of a piped water supply system in Nepal dates as far back at 1898, “Bir Dhara” was commissioned to supply water to the people of Kathmandu. A few water supply systems were installed in other towns of the country but practically no action was taken for the improvement of community water supplies up to the end of the nineteen sixties.

Till 1950, drinking water supply was limited to the urban areas of Kathmandu. Now most of the 33 urban centres in the country have piped water (CBS 1989). However, many supply systems provide water for only a few hours each day (ADB 1985) and despite receiving level of treatment bacteriological contamination remains high.

The total population having access to the piped water at the end of the decades of 70's, 80's and 90's were noticed to be 6%, 11% and 37% respectively. At present, the population having access to such piped water facility is estimated to be 70%. Although some rural water supply schemes exist, most of the rural population was traditional source of water, irrespective of quality (ADB, 1985). In rural areas as well, coliform contamination of drinking water is a major concern.

In early 1970, HMG of Nepal with the purpose to provide a water supply to the people established three active agencies which are:

a) Department of water supply and sewerage (DWSS)-responsible for providing drinking water supplies to all rural communities with more than 1500 population.
b) Ministry of Local Development (MLD)-responsible for providing drinking water supplies to all rural communities with more than 1500 population.
c) Water supply and sewerage Board (WSSB)-responsible for providing water supplies to tall the urban centers.

In 1980, only 6.6% (861,000) of the rural and 83% (687,000) of the urban population of the total population had house connections or easy access to a piped water supply.

The population in the hills and mid lands obtain water for their domestic and other uses from springs, ponds, canals, streams, river where as people in the Terai get water from privately owned dug wells and tube wells. Generally, the wells are polluted due to poor construction improper usage. In the urban areas nearly 20% of the population has house connections, while 55% get water from stand posts. Since water is supplied only two times a day for about 4 to 8 hours,

those who have connections provide a tank in their house to stone water during the flow hours.

At present, Nepal and many other developing countries is launching the international drinking water supply and sanitation. Decade plant as proclaimed by the UNO. By 1990, the government of Nepal planned to provide potable water to 67% of the rural and 94% of the urban population.

Those urban centers where piped water supply exists receive treatment with bleaching powder or lime used for disinfection. But most of our rural communities, there is no such water disinfection system.

3.1.1.5 Study of Physico-Chemical Parameters of Drinking Water

Sharma (1986) studies chemical parameters of tap water samples from 51 localities in Kathmandu. He found that little variation was observed in the chemical content of drinking water supplied to different localities in Kathmandu. The PH content ranged from 6.5 to 7.5, while $Caco_3$ content varied from 26 to 30 mg/l. Total hardness due to presence of various metallic salts was also similar from one location to another. The chemical constituents tested were found to be within the standards prescribed by (WHO, 1984).

CEDA (1989) carried out similar studies from 13 sites including three reservoirs-Balaju, Maharajgunj, Mahankal Chour. The chemical parameters tested including pH, Dissolved Oxygen (DO) Biochemical Oxygen Demand (BOD), chemical Oxygen Demand (COD), Ammonia (NH_3), Nitrate (NO_3), Phosphate (PO_4) and chloride (Cl).

DISVI (1989) a detail chemical analysis of drinking water which corrode pipelines, affect the aesthetic quality of water, and indicate contamination by domestic sewage. The DISVI study covered treatment plants and reservoirs, hospital storage tanks and tape in the public water supply systems.

DISVI (1990b) studied in seven rural areas of Illam in Eastern Nepal found that water samples from six sources were acidic with pH values outside the acceptable limit set by WHO. Total hardness and chlorides were also not within the desirable level. Other parameters such as conductivity, Ammonia (NH_3-N), Nitrate (NO_3-NO), Manganese (Mn), Iron (Fe) and Phosphate (PO_4) were found to be within the WHO standards.

DISVI (1990C) studied in seven rural areas in the Eastern Development Region of Morang, Sunsari, Jhapa, Siraha and Saptari and found that chemical parameters of most of the water samples within WHO standards for drinking water quality study found that only 3 percent of the samples collected from project tubewells and none of the samples collected from project other sources were above WHO guidelines.

3.1.1.6 Treatment of Drinking Water in Developing Countries

The earliest recorded knowledge of water treatment is in the Sanskrit lore. Sanskrit writings dating about 2000 B.C. tell how to purify foul water by boiling it in copper vessels, exposing to sunlight, filtering through charcoal and cooling in earthen vessels. The English philosopher, Sir Francis Bacon, published his experiments on the purification of water by filtration, boiling distillation and clarification by coagulation. He also remarked that the clarified water tended to improve health and increase the pleasure of the eye. Lue Antonio Porzio, an Italian Physician, first published the description of the sand filter in 1685. Perhaps, this filtration method is the first work on mass

sanitation. Later this method of filtration well applied all over the Italy (Clark, Viessman and Hammer, 1977).

Now the search for better living conditions and development of new technologies in water treatment has brought many sophisticated water treatment methods into practice. However developing countries are not able to enjoy these improved, advanced technologies.

The selection of water treatment methodologies is depended on factors like type of source, characteristics of water, degree of finished water, quality, geographical location, local conditions and socio-economic conditions. Depending on these factors, the application and combinations of treatment units are variable in all countries including the developing ones.

Filtration is the most commonly used technique for water purification in developing countries; Dual media filters (coal-sand) are used in several Latin American Countries India and Thailand were coal is available. It does a very good job and is cheap as well. Now a days flocculators and clarifiers are also extensively used in developing countries (Ranjitkar, 1985)

3.1.1.7 Treatment of Drinking Water in Rural Community of Nepal

Most of our rural communities are depending on piped water supply disinfection or treatment of Drinking water has never been done for moral of contamination in rural communities. Some localities even do not have water sources for drinking water that people collect water from sources away from point of use.

The fundamental purpose of water treatment is to protect the consumers from pathogens and impurities in the water that may be offensive or injurious to human health. The purpose of water treatment is to provide water with most

suitable composition. The major water treatment processes employed in practices are: filtration, ultra filtration and reverse osmosis, sedimentation, flocculation, adsorption, ion-exchange, addition of conditioning agents or additives, degasification, oxidation and disinfection. The selection of the appropriate system for water treatment includes different factors including requirements and preferences of the users, laws and regulations treated water availability requirement and finally minimum total cost comprising capital investment, operating costs, space, requirements, operating material usage, water and energy consumption (WHO, 1993.)

Urban treatment of water is similar globally and consists of steps pre disinfection, coagulation, sedimentation, filtration and disinfection etc. Those rural consumers who do not have piped water supply and disinfection treatment consume water directly from natural sources and need conventional method to rid water of disease carrying germs, can take a long time and use up of a lot of fuel.(IWTC,1990).

Rapid and slow sand filtration methods are also simple techniques which can be used in rural communities but these are not in common practice. Before selecting any treatment process for disinfection of drinking water, economic status of community people should be revalued. Most of our communities are below poverty line thus people even do not take interest to afford money for water treatment because they are happy to consume contaminated and polluted water unknowingly, thus, community people need a cheap reliable and simple treatment method for drinking water.

CHAPTER-IV

4. MATERIAL AND METHODS

4.1 MATERIALS

Lists of materials used during this study are following:

1) Conical flask
2) Glass rods
3) Slide
4) Loops
5) Microscope
6) Incubator
7) Freeze
8) BOD Freeze
9) Burette
10) Burette stand
11) Weighing meachine
12) Pipettes
13) Beakers
14) BOD Bottles
15) Test Tubes
16) Petri plates
17) Cotton Plug
18) Burner
19) NA (Nutrient agar)
20) MA (Mac Conkey agar)
21) S-S agar
22) M-Endo agar
23) Selenite-F-Broth
24) Mac Conkey Broth
25) Biochemical Media

4.2 Sampling and Sampling Sites

Paper wrapped and pre-sterilized for 15 lbs (121°c) for 15 minutes sampling bottles were used for sampling. The water samples were taken from different sources, leaving a small space in the sampling bottle after sample collection. While sampling in natural waters, bottle was lowered in water at a depth of 15 to 30 cm in the water, and tilted the need slightly upwards to let it fill completely keeping a small space before replacing the cap. In case a tap water, external fittings from the tap were removed and tap was sterilized using alcohol and ignited. Then tap was allowed to cool by running the water to waste for 2 minutes. Sample bottle was filled from a gentle flow of water and cap of the bottle was replaced. A code number, Sampling date and sampling time was written to each bottle using a marker.

Following seven water sources sites were selected for the study

S_1 = Sundharighat river

S_2 = Sundharighat Canal

S_3 = Sundharighat Tank

S_4 = Balkhu Tap Water

S_5 = Kuleshwor tap water

S_6 = River water sample

S_7 = Stream water sample

4.3 Sample Transportation and Preservation

Water samples after collection should be carried to laboratory as soon as possible. If microbial examination is not possible within one hour the samples should be keep in refrigerator. Sometimes if required samples should be preserved at 4°c up to 6 hrs. In this study samples was subjected to microbial examination within four hour of collection .The Sample were transported by keeping them in an the box at 4°c.

4.4 Standard Microbiological Methods for Drinking Water Quality Assessment

4.4.1 Most Probable Number (MPN) Method

The technique involves inoculating the sample in a suitable liquid medium and incubating for suitable period, the tubes were examined for gas production by the coliform organisms. This test is known as the presumptive test. Since this reaction might also be produced by the organisms other than the coliforms. The positive tubes from the presumptive test were subjected to a confirmatory test for a definite presence of coliform bacteria, the completed test is named out for confirmatory test, Brilliant Green Lactose bile broth is used and for completed test. EMB (Eosin Methylene Blue) or M-end agar media is widely used. Eijkman's test was done for confirmatory defection of faecal coliforms.

For each sample nine tubes with MacConkey broth were prepared 3 tubes contained 10 ml of double strength MacConkey broth and 6 tubes contained 5 ml of single strength MacConkey broth. Then all of these tubes sterilized 10 ml of water sample was inoculated into 3 tubes of double strength, 1 ml and 0.1 ml of samples were inoculated into 2 sets of tube and change in colour from red to pinkish was observed after incubation at 37°c for 24 hours with help of MPN Chart, total coliform in water was estimated.

4.4.2 Membrane Filter Method

All the water samples were filtered through pre-sterilized Millipore filter apparatus then the Millipore filter was transferred into plate containing M-Endo agar and incubated at 37°c for 24 hrs production of greenish metallic sheen colonies were observed.

4.4.3 Serial Dilution of Sample

a) One ml of water sample was taken and transferred to tube containing 9 ml. of sterile distilled water. This became 10^{-1} diluted.
b) Similarly 1 ml. at 10^{-1} diluted sample was taken and transferred to another tube containing 9 ml of sterile water. In similar way serial dilution was performed up to 10^{-6} dilution.

4.4.4 Pour Plate Method

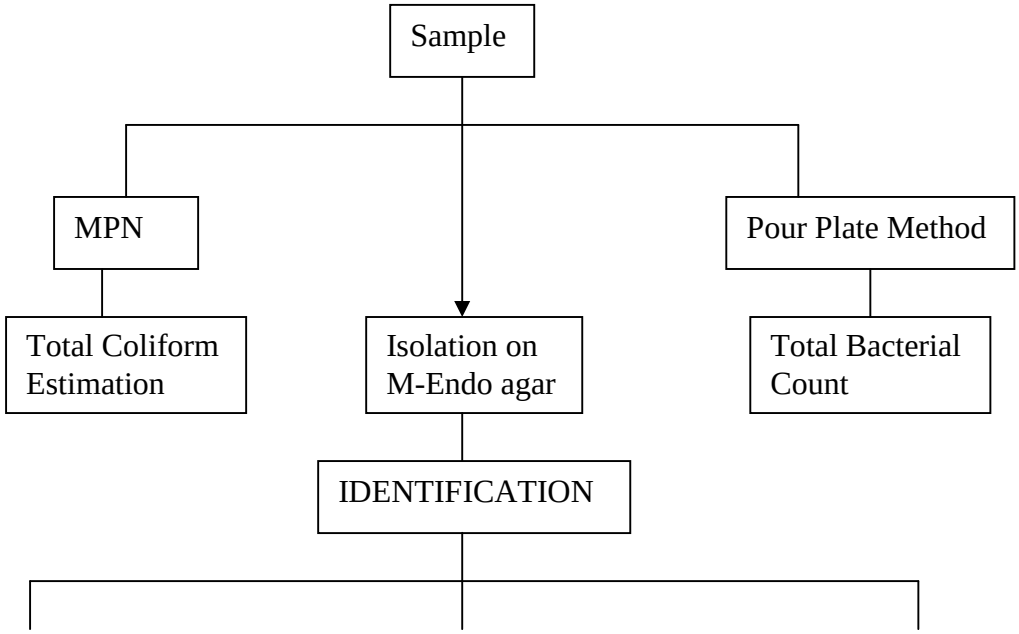
a) From each dilution 1 ml of diluted sample was taken in peptriplate and molten, cool Nutrient Agar (NA) media was poured.
b) The sample was uniformed mixed by rotating the plate and allowed to solidify.
c) Plates were incubated at 37°c for 48 hours and number of colonies were counted.

4.4.5 Identification of Bacteria

Bacteria isolated in respective selective or differential medium were identified by standard microbiological method following Bergey's Manual of Systemic Bacteriology (1986). The isolated bacteria were identified on the basis of Colonial characteristics, morphological characteristics and biochemical properties.

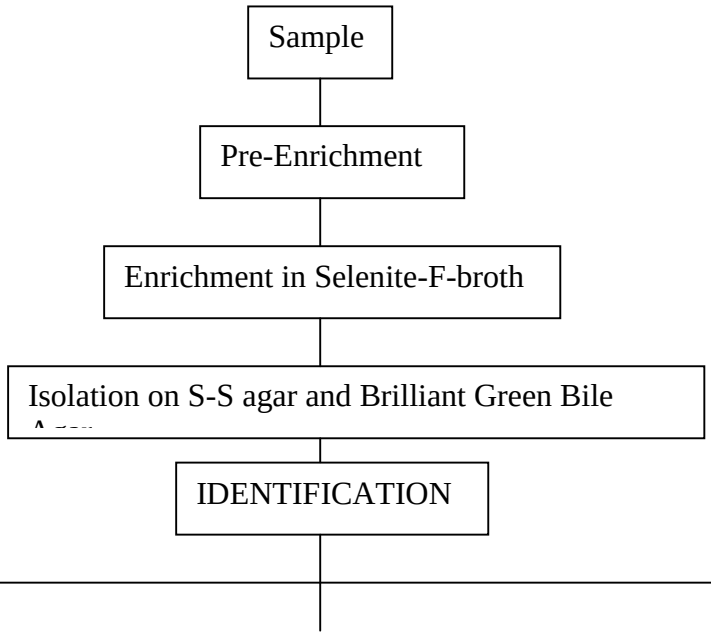
Morphology, Colonial characteristics and biochemical characteristics of bacteria is given in flow chart (Mackie and Mac Cartney 1989; Manual of food Microbiology, FAO, 1992).

Chart 1: Flow chart for Identification of *Escherichia coli*



Cultural Character	**Morphology**	**Biochemical Test**
Pinkish Red Lactose Fermenting Colonies in Mac-Conkey agar	Gram –ve nonsporing bacilli	Catalase + Oxidase – Glucose (gas) + Lactose (acid) + Indole + Methyl Red + Voges proskaur – Citrate – Urease – H_2S test – Gelatin Liauefaction -

Chart 2: Flow chart for Identification of *Salmonella species*



Cultural Character	**Morphology**	**Biochemical Test**
Salmonella Colony appears as pinkish white surrounded by bright red medium on brilliant green bile agar. On S-S agar colonies are colourless H_2S producer give Black centers.	Gram –ve bacilli nonsporing, non capsulated most species are motile	Catalase + Oxidase - Glucose (acid) + Lactose (acid) - Sucrose (acid) – Indole - Methyl Red + Voges proskaur – Urease – H_2S +/- Citrate

4.4.6 Detection of *Salmonella*

a) 5 ml of each water sample was separately mixed in 45 ml of selenite broth.

b) Then media was incubated at 37°c for 24 hours.

c) After incubation one loopful of suspension was taken and streak on S-S agar media and plate was incubated at 37°c for 24 hours.

d) Suspected salmonellae (black centered colonies) were sub cultured on NA and identified by biochemical test.

Morphological Study

Colonial Characteristic: All the isolates grown on nutrient agar for 24 hours were studied for their colonial characteristic. Since the most organism showed good growth at $37^{o}C$, all the test for identification process were carried out at $37^{o}C$, as described in Bergeys manual, 1986.

Cell Morphology

Cell Morphology was studied using gram's reaction. The morphology was studied under oil immersion.

4.4.7 Biochemical Tests

Appropriate biochemical tests were done for Identification of the bacteria. The isolated pure colonies were inoculated on to different bio chemical medium for the test as shown in table 1.

Table 1. Biochemical Tests performed for Identification of *Enteric Bacteria*

S.N.	Biochemical Medium	Tests
1.	3% Hydrogen Peroxide	Catalase
2.	1% Trimethyle paraphenylelediamine dihydrochloride	Oxidase
3.	Glucose Phosphate broth	Mixed acid Fermentation
4.	Peptone water	Indole
5.	Simmon's Citrate	Utilization of citrate
6.	Triple Sugar Iron agar	Acid Fermentation
7.	Urea Agar	Urease

Composition and preparation of media used for isolation and identification of bacteria and detail of Gram's staining procedure and Gram's reagent is given below.

Catalase Test

Catalase test is done to test the presence of enzyme catalase. The enzame catalase splits hydrogen peroxide to water and oxygen.

Reagent: 3% hydrogen peroxide.

Composition

Concentration hydrogen peroxide	3ml
Distilled water	97ml

Procedure

Three ml of 3% hydrogen peroxide was taken in a test tube and colony of bacteria to be tested was picked up from nutrient agar with the help of glass rod and inserted into the tube containing reagent. The production of gas bubble immediately indicates positive catalase test.

Citrate Utilization Test

Citrate Utilization test is performed to determine if an organism is capable of utilizing citrate as the sole source of Carbon for metabolism with resulting alkalinity.

Medium – Simmon's Citrate Agar

Composition	(gm/Litre)
Monoammonium phosphate	1.0
Dipotassium phosphate	1.0
Sodium Chloride	5.0
Sodium Citrate	2.0
Magnesium Sulphate	0.2
Bromothymol blue	0.08
Agar	15.0
Final pH (at 25ºC)	6.8 ± 0.2

Preparation

24.2 Grams was suspended in 1000ml distilled water. It was heated to boil to dissolve the medium completely. It was distributed in tubes and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. The medium in tubes were solidified in slanted position.

Procedure

The Slant was streaked with test organisms and incubated at 37ºC for 48 hours. Growth of organism with an intense blue colour on slant is the indicative of positive test. No growth no change in colour (green) is the negative test.

Hydrogen Sulphate Test (Triple Sugar Iron Agar Test)

The test is done to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium, with or without the production of gas, alongwith determination of possible hydrogen sulphide (H_2S) production.

Composition	(gm/Litre)
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Cretose	10.0
Suctose	10.0
Pextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol red	0.024
Agar	12.0
Final pH (at 25ºC)	7.4 ± 0.2

Preparation

6.5 grams was suspended in 100ml distilled water. It was boiled to dissolve completely. It was distributed in tubes and sterilized by autoclaving at 15 Lbs pressure (121ºC) for 15 minutes. The medium was allowed to set in sloped from with a butt about 1 inch long.

Procedure

The test organism was stabbed in the butt and streaked on the slant. The tubes were incubated at 37ºC for 24 hours. Black colouration of butt was indicative of H_2S formation. The change in colour of butt, slant and gas formation was also noted and recorded as alkali/alkali, alkali/Acid and acid/acid for the growth of a fementers, glucose fementers and all sugar fementers.

Indole Test

Indole test is done to determine the ability of an organism to split Indole from tryptophan molecule.

Medium – peptone water

Composition	(gm/Litre)
Peptone containing tryptophan	10.0 gm
Sodium Chloride	5.0 gm
Distilled water	1000.0 ml

Fifteen grams was dissolved in 1000ml distilled water. It was boiled to dissolve completely. It was dispended in test tubes and sterilized at 15 lbs pressure (121ºC) for 15 minutes.

Reagent : Kovac's reagent

Composition	(gm/Litre)
P-dimethyl aminobenzaldehyde	5.0 gm
Isoamyl alcohol	75.0 ml
Concentrated hydrochloric acid	25.0 ml

Aldehyde was added to flask containing alcohol and it was dissolved by gentle warming to 55ºC in a water bath. It was cooled and HCl was added. It was stored in a dark glass bottle in a refrigerator.

Procedure

The test organism was incubated in peptone water and incubated at 37ºC for 48 hours. About 0.5 ml Kovac's reagent was added and shaken gently. Formation of pink coloured ring over surface layer indicated positive test.

Methyl Red Test

The methyl Red Test is done to test the ability of an organism to produce and maintain stable acid products from glucose fermentation and to overcome the buffering capacity of the system.

Medium – MRUP medium (Glucose – phosphate broth)

Composition	(gm/Litre)
Buffered peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0
Final pH (at 25ºC	6.9 ±) 0.2

Seventeen grams was dissolved in 1000 ml distilled water. It was distributed in test tubes in 10 ml amount and sterilized by autoclaving at 15 Lbs pressure for 15 minutes.

Reagent – Methyl Red

Composition	(gm/Litre)
Methyl Red	0.04 gm
Ethyl alcohol (absolute)	40.0 ml
Distilled water	1000 litre

Methyl red was dissolved in Ethyl alcohol and water was added.

The glucose phosphate broth was inoculated with culture to be tested and incubated at 37ºC for 48 hours. Methyl red indicator was added to the culture and development of red colour indicated positive test while yellow colour indicates negative test.

Oxidase Test

Oxidase test is done to determine the presence of the oxidase enzyme. Oxidase reaction is due to the presence of a cytochrome oxidase system.

Oxidase reagent

What man No.1 Filter paper was cut into strips of 6-8 cm in diameter. It was soaked in the reagent till saturation. The paper strips were drained and freeze dried and stored in a dark tightly sealed bottle.

Procedure

The oxidase test paper was moistened with distilled water. A colony to be tested was picked using glass rod and rubbed to the paper. Development of violet colour within 10 seconds is an indicative of positive test.

Sulfide – Indole – Motility Medium (SIM)

Sulfide-Indole-Motility is a semi solid medium used for the determination of sulfide production, Indole formation and motility of enteric bacteria.

Composition	(Gram/Litre)
Breef extract	3.0
Peptone	30.0
Peptonized iron	0.2
Sodium thiosulphate	0.05
Agar	3.0
Final pH (at 25ºC)	7.3 ± 0.2

36.25 grams was suspended in 1000 ml distilled water. It was heated to boil to dissolve the medium completely. It was dispense in tubes and sterilized by autoclaving for 15 minutes at 15 Lbs pressure (121ºC). The medium was allowed to solidify in a vertical position.

Procedure

The test organism was stabbed into the medium and incubated at 37ºC for 24 hours. Motile organism show diffuse growth or turbidity away from the line of inoculation and non motile grows only along the line of inoculation. H_2S positive test is indicated blackening along the line of inoculation.

0.2 ml of Kovac's reagent was added to the tube and allowed to stand for 10 minutes. A dark red colour in the reagent indicates a positive indole test.

Urease Test

i) Urease test demonstrates the ability of an organism to split forming two molecules of ammonia by the action of the enzyme urease.

ii) 24 grams Urea agar base was suspended in 950 ml of distilled water. It was boiled to dissolve completely and sterilized by autoclaving at 15 lbs pressure (115ºC) for 15 minutes. It was cooled down to 55ºC and aseptically introduced 50ml of sterile 40% Urea solution and mixed well. It was distributed in sterile test tubes and allowed to solidify in slanted position.

Fresh culture of test organism was streaked heavily on the slant and incubated at 37ºC for overnight. Change in colour of medium to pink indicate positive test and no change in colour indicate negative test.

Medium - Urea agar base.

Composition	(Gram/Litre)
Peptone	1.0
Dextrose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Monopotassium phosphate	0.8
Phenol Red	0.012
Agar	15.0
Final pH (at 25ºC)	6.8 ± 0.2

Voges – Proskauer Test

i) Voges – Proskauer test determines the ability of organism to produce a neutral end product, acetylmethylcarbinol from glucose fermentation.

ii) Procedure

Sterile borth was incubated with fresh culture medium and incubated at 37^{o}C for 48 hrs. Development of pink-red colour withih 30 minutes after adding of @-napthol and 40% potassium hydroxide in 1:3 proportions was recorded as positive test.

Medium – MRVP medium (Glucose – phosphate both)

Reagent – Baritt's reagent

Solution A

@- naphthol	5.0gm
Ethyl alcohol (95%)	100.0ml

Solution B

Potassium Hydroxide	40.0gm
Distilled Water	100.0ml

4.4.8 Physico-Chemical Parameters of Water

Analysis of water samples were done by following "Standard methods for examination of water and waste water "(APHA 1995). The recording of pH, temperature and analysis of DO were carried out in the field. The test for nitrate, phosphate, sulphate, total hardness, BOD were determined in the laboratory during the investigation period.

Temperature

The surface temperature of the water was recorded directly by dipping the bulb of an ordinary mercury thermometer in the water. The temperature was taken on the centigrade scale.

pH

pH of the water samples was recorded with pH paper in the field and with pH meter in the laboratory.

Dissolved Oxygen (DO)

For the determination of dissolved oxygen, BOD bottle was filled with water sample. During placing the stopper on the bottle, care was taken to avoid trapping air in the bottle. In the bottle, 2ml of manganese sulphate solution was added at the bottom and 2ml of Alkaline KI solution was added at the internal of few seconds with the help a separate pipette after removing the stopper. The stopper was put to exclude air bubbles and mixed the solution by inverting the bottle for few times. After the precipitate had settled sufficiently 4ml of conc. H_2SO_4 was added to it. The stopper was put carefully and inverted the bottle for several times until the dissolution of precipitate was completed. 100ml of sample from BOD bottle was taken in a conical flask and titrated against sodium thiosulphate solution, $Na_2S_20_3$(0.025N) until a light brown color appeared. Few drop of

starch solution was added as a indicator and again titrated against sodium thiosulphate until the blue coloured appeared. The volume of titrant used in the getting the end point was noted which is nothing but a quantity of dissolved oxygen. These reading were noted and mean was taken which was converted into mg/ltr by a following sample equation:

$$\text{Dissolved oxygen, mg/l} = \frac{\text{(ml x N) of titrant x 8 x 100}}{V_2 \quad \frac{(V_1\text{-}V)}{V_1}}$$

Where,

V_2= Volume of the sample taken for titration

V_1=Capacity of bottle

V= Volume of $MnSO_4$ and Alkaline KI used

Biochemical Oxygen Demand (BOD)

For the determination of BOD, 2 BOD bottles (300ml) were filled with water sample .One BOD bottle was taken for the calculation of $D0_1$ as described in DO. Another BOD bottle was taken for calculation of DO_5. For the determination of DO_5, BOD bottle with sample without any air bubble was incubated at 20°c for 5 days. After 5 days incubation at 20°c, same method was followed to calculate DO_5 as for DO_1 given above. Wrinkler's titrimetric method was followed for the determination of DO_5

Hence, BOD can be calculated as follows-

BOD Mg/l = $(DO_1\text{-}DO_5)$* Dilution factor

Where,

DO_1 = Initial DO in the Sample

DO_5 = DO after 5 days.

Total Hardness

Total hardness of the water sample was estimated by EDTA Titrimetric Method for this 50 ml of sample was taken in a clean conical to which 2ml of ammonia buffer solution was added and stirred for through mixing. To this 200 mg of Erichrome Black T indictor was added and shaken well. Then the sample was titrated against standard EDTA solution of 0.01 N till the colour changed form wine red to blue. The Volume of EDTA consumed was noted. Three readings were noted and mean was taken which was converted into mg/l by using following equation.

$$\text{Total hardness } CaCO_3, \text{ Mg/l} = \frac{\text{ml EDTA used X1000}}{\text{ml Sample}}$$

Nitrate (NO_3)

First a standard Calibration curve containing concentration and absorbance were prepared with standard nitrate solution (Mackereth *et. al*, 1978), then the concentration of nitrate in the water. Sample was measured following the phenol disulphonic method for this 50ml of filtered sample was taken in a conical flask and an equivalent amount of silver sulphate solution was heated slightly the precipitate of AgCl. Then, filtrate in the porcelain basin was evaporated to dryness on a stean bath. The residue was cooled and diluted the contents to 50ml with double distilled water. To this content, 6ml of liquid ammonia was added to develop the yellow colour. A reagent blank was also made by adding phenol disulphonic acid and liquid ammonia to doubled distilled water and the volume made to 50ml. The absorbance of sample and the reagent blanks was measured at by spectrophotometer within 3 minutes of colour development. The concentration of nitrate n the sample was determined form the standard calibration curve.

Nitrite (NO_2)

A standard calibration curve containing concentration and absorbance were prepared with standard nitrite solution. Then the concentration of nitrite in water sample was measured using sulphanilic acid for this 50 ml of the filtered sample was taken in a conical flask and 1 ml of each EDTA, Sulphanilic acid, 2-napthylamine hydrpchlride and sodium acetate solutions in sequence. The solution was kept for a moment and colour development was measured by reading the absorbency at 520nm by spectro photometer.

Phosphate

Like the nitrate, a standard calibration curve of orthophosphate was also prepared with standard phosphate solution followed by Mackerth et. al. (1978). A stannous chloride method was used to determine the concentration of orthophosphate for this 50 ml of the filtered water sample was taken in a conical flask and a few drops of phenolphthalein indicator was added method. If the sample developed any colour was neutralised by drop wise addition of strong acid solution. Then 2ml of ammonium molybdate was added in the sample followed by 5 drops of stannous chloride solution. The sample was shaken well and kept for 5 minutes to develop a blue colour. This was measured by the spectrophotometer at 690nm against a distilled water blank with the same amount of the chemicals. The level of orthophosphate content was calculated using the calibration curve.

Sulphate (SO_4)

Sulphate is calculated by using Turbidimetric method. The sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The concentration of sulphate can be determined from the absorbance of the light by barium sulphate and then comparing it with a standard curve.

100 ml of water was taken in a conical flask and 20 ml of buffer solution was added and mixed in stirring apparatus. While stirring a spoonful of Bacl2 crystals was added and stirred only for 1 minute at constant speed. After stirring period has ended, Solution was poured into absorption cell of spectrophotometer and turbidity was measured at 420nm exactly after 4 minutes. Concentration of sulphate was found out from the standard curve.

Chloride

For chloride, 50 ml sample was taken in a conical flask and 2 ml of K_2CrO_4 solution was titrated against $AgNO_3$ (0.02 N) until a persistent red ring appears. The Volume of $AgNO_3$ consumed was noted. Three readings were noted and mean was taken which was converted into mg/l by using following simple equation.

$$\text{Chloride mg/l} = \frac{(\text{ml} \times \text{N}) \text{ of } AgNO_3 \times 1000 \times 35.5}{\text{ml of Sample}}$$

CHAPTER-V

5. RESULTS

5.1 Physico-Chemical Analysis of Water

Water samples were brought to the laboratory in icebox and immediately inoculated for the bacteriological study and tested for the physico- chemical parameters.

Temperature was noted at the sampling sites of all water samples using thermometer. Temperature of the water samples were found approximately near value depending up on the season of sample collection. The temperature of all water sources found to be nearly equal as shown in table 2.

Table 2: Results of Chemical Analysis of Water Samples.

SN	Parameters	Sample number							WHO Guidelines
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	
1	Temperature (^oC)	17.0	16.0	16.0	14.0	15.0	14.0	17.0	*
2	pH	7.42	7.10	7.08	7.60	7.80	7.62	7.80	7.0-8.5
3	Total Hardness (as $CaCO_3$- mg/l)	125.0	160.0	50.0	160.0	180.0	170.0	140.0	300.0
4	Nitrogen-Nitrate (mg/l)	0.40	0.16	0.15	1.80	1.40	1.60		10.0
5	Nitrogen-Nitrite (mg/l)	0.14	-	-	0.18	0.20	0.28	0.20	3.0
6	Phosphates (mg/l)	0.50	0.16	-	0.25	0.25	0.54	0.24	*
7	Sulphates (mg/l)	18.0	48.0	12.0	75.0	60.0	88.0	12.0	150.0
8	Chloride (mg/l)	250.0	300.0	140.0	250.0	150.0	210.0	250.0	200.0
9	Dissolved Oxygen (DO) (mg/l)	8.20	5.40	6.80	4.42	4.20	3.88	6.40	*
10	BOD (mg/l)	3.20	2.80	2.88	8.80	11.20	8.86	2.66	25.0

S_1 = Sundharighat river S_2 = Sundharighat Canal

S_3 = Sundharighat Tank S_4 = Balkhu Tap Water

S_5 = Kuleshwor tap water S_6 = River water sample

S_7 = Stream water sample * = No health based values has been proposed

\- = upto reported limit, concentration of parameter not detected

pH was noted at the sampling sites using pH paper and at the laboratory using pH meter. The pH values of water samples were found around pH 7 which was which was shown in table 2.

Dissolved oxygen (DO) of the water was fixed at the sampling site and estimated by Wrinkler's titrimetric method. Stream Sundharighat river sample found high DO value 8.20 and stream water sample found DO is 6.40 and Sundharighat tank found to be 6.40 but low value of DO found as 4.20 from Kuleshwor tap water.

Biochemical Oxygen demand (BOD) was calculated following Wrinkler's titrimetric method and found to be 8.6 from river water sample of 8.80 from Balkhu tap water but high number from Kuleshor tap water was 11.20 and low. BOD value was 2.66 from stream water sample.

Other parameters like total hardness, nitrates, phosphates, sulphates and chlorides were detected in very small quantity and found below the WHO tolerance limit. Similarly nitrite was not reported up to detectable limit from some water samples and found to be very low. Similarly negligible value of nitrates and phosphates were reported from water samples. Chemical parameters detected are given in table 2.

5.2 Bacteriological Quality.

Seven water samples were tested for *coliforms* and maximum amount of *coliform* were observed from all water samples and *coliform* count was done by most probable Number.

The highest coliform count was found in Sundharighat River, and total Bacterial count was found in River water sample and lowest bacterial count was found to be 1.00×10^4 in Sundharighat tank.

Table 3: Microbial Analysis of Drinking Water Samples.

SN	Sample No. (Sources)	Total Bacterial Count (CFU/ml)	Total Coliform Count	Detected micro-organisms from MPN	Detected micro-organisms from Pour Plate Method
1	S_1	$4.00X10^6$	900	*E-Coli*	*E-Coli, Salmonella sps*
2	S_2	$6.00X10^4$	930	*E-Coli*	*E-Coli*
3	S_3	$7.00X10^4$	500	*E- Coli*	*E-Coli*
4	S_4	$5.00X10^6$	800	*E-Coli*	*E-Coli*
5	S_5	$3.00X10^3$	700	*E-Coli,*	*E-Coli*
6	S_6	$6.40X10^6$	1100	*E-Coli*	*E-Coli, Salmonella sps*
7	S_7	$4.00X10^6$	500	*E-Coli*	*E-Coli*

S_1 = Sundharighat river S_2 = Sundharighat Canal

S_3 = Sundharighat Tank S_4 = Balkhu Tap Water

S_5 = Kuleshwor tap water S_6 = River water sample

S_7 = Stream water sample

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

This study was undertaken with an aim to investigate drinking water and river water quality of Kathmandu. Drinking water samples were analyzed for bacteriogical quality of different water sources such as Sudharighat River, Sudharighat canal, Sudharighat tank, Balkhu tap water, Kuleshor tap water, River water sample and stream water sample of Kirtipur area. *Escherichia coli* and *Salmonella* Standard Plate Count were investigated in those water samples.

Water is a vital resource for humans, but it can be extremely dangerous when it becomes the vehicle of transmission of diseases and causes explosive out breaks of such diseases which may be widely disseminated. Many studies have implicated the poor water quality as a principal factor for mortality and morbidity associated with enteric diseases. Similar cases of typhoid, cholera and gastro-enteritis illness were found closely related to poor water quality. In developing and under developed countries, 40% of deaths are estimated to occur among children under 15 years old, of which leading cause is due to diarrhea and parasitic diseases. An estimated 30,000 were during everyday, many of them from diseases attributable to a lack of safe water or adequate sanitation facilities.

In this study, chemical quality of water samples from all seven sources were found to be satisfactory and were within the acceptable limits set by WHO (1993). Several parameters were tested to determine the physico-chemical quality of water and most were within WHO standards for drinking water except BOD value of some samples. Temperature, PH, Total hardness, Nitrate,

Phosphate, Sulphate, Chloride that may affect the aesthetic quality of drinking water. Nitrite was not reported detectable limit from some water samples. Similarly, negligible value of nitrate and phosphates were reported from water samples. Likewise chloride and total hardness were reported within the safe limits set by WHO (1993).

DO value of Sundharighat river and stream water samples were high while River water sample, Balkhu tap water were low in all water samples. Higher value of DO it was 8.20 mg/l in sample Sundharighat River while low in river water sample was 3.88 mg/l.

Similar results were obtained by Sharma (1986) and he reported that most tap water samples from 51 localities in Kathmandu were chemically suitable for drinking purposes. The PH content ranged from 6.5 to 7.5 while $CaCo_3$ content varied from 140 to 300 mg/l. Total hardness due to the presence of various metallic salts was also similar from one location to another. The chemical constituents tested were found to another. The chemical constituents tested were found to be within the standards prescribed by WHO (1984). Similarly in this study also, almost all water samples are chemically suitable for drinking purposes.

The hardness of water depends on the combination of carbonates of calcium and magnesium. The calcium content of the water bodies fluctuates directly with bicarbonates and both of these moves inversely with carbonates and PH. The direct relationship of calcium and bicarbonates has also been observed by Pearshal (1953). Hardness is also an indicator of pollution effected by urine, sewage and manure of and geochemical nature of water sources.

Chloride is abundant in nature. In surface water, Chloride in high concentrations is an indicator of faecal pollution. Similarly higher phosphates

value indicates faecal pollution from sewage, Manure, fertilizer, pesticides and detergents.

Organic and inorganic Nitrogen compounds come form different sources like precipitation, scours and agricultural fields and sewage. Nitrate is a sign of faecal pollution in a concentration of 0.2-2 mg/l (WHO, 1993). In unloaded waters no nitrites would be found. Nitrate was produced from Nitrite by Nitrification of organic matter, disposal of sewage and industrial effluent and fertilizer from adjacent land.

The concentration at which water constituents are offensive to consumers is dependent on individual and local factors, including the quality of the water to which the community is accustomed and a variety and a variety of social, economic and cultural considerations. A worldwide survey found that among the 1600 chemicals detected in drinking water, 22 caused cancer, 42 were suspected of causing cancer, 27 promoted tumors and were pro-cancer, 50 caused birth defects and 15 caused some mutation. (IWTC, 1990). Under these specific to substances that affect the acceptability of water to consumers.

The health risk due the chemicals exceeding tolerance limit in drinking water differs from that caused by microbiological contaminants. There are few chemical constituents. There are few chemical constituents of water that can lead to acute health problems. The fact that chemical contaminants are not normally associated with acute effects places them in a lover priority category than microbial contaminants. The effects of which are usually acute and widespread.

The health risk due the chemically exceeding tolerance limit in drinking water differs from that caused by microbialogical contaminants. There are few chemical constituents of water that can lead to acute health problems. The fact

that chemical contaminants, the effects of which are usually acute and widespread.

The maximum count of bacteria was observed from all sources is Sundharighat river, S.Cannal, S.tank, Balkhu tap Water, Kuleshwor tap water, River water sample and stream water sample. Highest bacterial count observed from River water sample, 6.40×10^{6}

Similarly, *coliform* count was higher in all water samples. Total *coliform* count was higher is Balkhu tap water River water sample and Khleshwor tap water Sundharighat river. The maximum *coliform* count was 1100, 930 and 900 cells per 100 ml from S_6 S_2 and S_1 samples respectively and lower *coliform* count was found to be in S_3, S_4, S_5 andS_7 samples.

Similarly Study was performed by Adhikari *et. al*.(1986) and reported that most of the water samples from taps, natural springs and ponds had more than 1.800 coli form per 100ml of water. 42 of the 48 tap water samples, 22 of the springs and 24 of 25 pond water samples were unsatisfactons, similar to the results of this study.

Results of this study was also supported by findings of Joshi(1987) in which be carried bacteriological tests of drinking water sources of two villages of central Nepal which was near to sampling sites of this study, chaubas (shivapuri) and syabru. He reported the coli from count ranging from 5-100 cells per 100 ml of water. Covered springs in chaubas water from covered springs showed coli from contamination within the range of 5-10 cells per 100 ml. Where as in syabru water from uncovered springs showed contamination within the range of 20-100 cells per 100 ml. In this study also coli forms count was maximum from spring water samples.

Similar results were observed in one of the DISVI (1990 b) study which carried out microbiological tests of drinking water in seven rural areas of Ilam in Eastern Nepal. The study tested the samples from springs, spring well, aquifers, rivers and river water reservoir and reported that all the sources had unacceptable levels of faecal *coliform* bacteria ranging from 2,400 cells/100ml. In this study also all water sources were found to contain unacceptable levels of faecal *coliform* bacteria. The main objective of the DISVI'S assignment was to generate baseline data on water and water quality at sources and households in order to calculate the impact of a health and sanitation education programme run in the villages.

ENPHO/DISVI (1990) investigated the bacteriological quality with similar objectives of this study, of ground water in Kathmandu. Samples were collected from stone spouts from 21 localities and reported that water from all the spouts was found to be facially contaminated. In their study, 81 percent of the sample contained an average *coliform* count of more than 100 cell /100 ml water. In this study also found to be faecally contaminated in this study.

Similar results were found by DISVI (1990) which assessed the ground water quality in seven rural areas of the Eastern Development Region of Nepal. DISVI reported that quality of water from tube wells was found to be far better than that of water from dug wells. About 90 percent of the samples from project tube wells had less than 10 faecal Coli forms cells/100ml, 8 percent had 100 cells/100 ml and 2 percent had more than 100 cells/100 ml All samples from non-project dug-wells hand more tha 100 cells/100 ml. In our study also none of the ground water samples were found to be better bacteriologically and found to have maximum number of *colifrom.*

All of these studies clearly indicated that most of the rural water sources are faecally contaminated, This indicated that the load of *coliform* bacteria in

natural water sources were higher than the WHO guideline Values .The probable source of contamination around natural water sources are unhygienic environment, Unmanaged sewage disposal, dust and dirt in the surroundings and recreational activities. At a number of areas faecal matters from baby diapers, Kitchen wastes and animal excreta accumulate at the public tap during washing and cleaning activities.

In most of the rural communities, people continue to use the most inconvenient sources of water irrespective of quality. Collected water is contaminated both outside and within the domestic environment through poor hygiene and sanitary practices. In most of rural people use to discharge excreta near by water sources making water contaminated. Village people are Unknown about the quality of water and consuming it and regularly facing the problems created by water borne infections.

Many villages Communities meet their water demand supplement with ground water sources. Ground water is extracted through bore and wells. Use of tube wells and dug wells is wide spread in area where is no easy access to surface water Ground water extracted from deep, well-protected aquifers are usually free from pathogenic micro organisms and the distribution of such untreated ground water is in common practice in many countries. Unsanitary and recreational activities around ground and microbial contamination of ground water sources.

In the communities studied, drinking water comes mainly from surface water sources i.e. streams, releruoirs and ground water sources i.e. wells and springs Generally streams, rivers and other surface water bodies are important for re creation and other surface bodies are also the main repository for the untreated sewage, solid waste and effluents. As the population increases and human use of surface water courses and bodies intensifies, many of these uses become

incompatible, thus high number if organisms are present in surface water sources and surface water will require usually full treatment.

The total bacterial count was found higher than WHO guideline in all samples. Total coli form count in Sundharighat tank (S_3) was $7.0X10^6$ CFU/ml which was higher than other samples but lowest number of Coli form count was 500 CFU/ml in Sundhanrighat tank (S_3). Similarly study was performed by Sharma (1986) and similar results were observed.

During rainy season, Volume of water bodies and flow of surface water sources increases. Water bodies become contaminated due to seepage of sewage, wastes and run-off the adjacent land. Simultaneously total bacterial Local and total *coliform* count increases along with pollution. During Dry summer season, water volume decreases and contamination level increases, increasing the bacterial load. An explosive outbreak of enteric diseases and epidemic occurs during the dry summer season and rainy season. During winter season low bacterial count and *coliform* count are found due to decreased level of pollution (IVC, 1990).

In this Study, a number of pathogenic enteric bacteria were isolated and detected from different water sources. Coli form bacteria such as *E.Colis Klebsiella species* and pathogenic bacteria such as *Salmonella species* were detected. These bacteria were given importance in accordance with public health of the people. *Escherichia coli* was detected in all water samples from different sources. While *Salminella sps* and *Klebsiella sps*. were detected mainly from Sundharighat river (S_1) and river water samples (S_6) .

Similar study was conducted by many workers and presence of coliform bacteria from water was reported. Likewise Sharma (1993) isolated

Escherichia Coli, Salmonella sps, Klebsiella sps, Proteus sps, Shigella Sps from water of different parts of the country.

The presence of high *coliform* bacteria and *Escherichia coli* is the indicator of faecal contamination. High number indicates unsanitary practices and poor hygienic condition of natural water sources. *Coliform* bacteria inhabits the intestinal tract of human and animals. Generally its presence in drinking water indicates faecal contamination although not all *coliform* are of faecal origin. *Coliforms* include all aerobic and anaerobic non-spore farming bailli such as *Escherichia coli, Citro bacter freundii, Enterobacter, Aerogens, Enterobacteriaceae* and *Klebsiella pneumoniae*. The presence of coliform micro organisms in drinking water may indicate the presence of bacterial infections that cause water borne diarrhea diseases (ADB, 1985).

In rural Communities, People use to collect water from sources away from point of use or store water in unsanitary condition in the house hold. Even water sources are free from contamination; house hold may be sources if contamination if not properly cleaned of maintained. Thus water is subjected to contamination in the house hold and to those may often bet the most supplies have served the people, Such sources of water must be adequate protected against pollution otherwise people health risk increases along with pollution.

A high incidence of enteric diseases associated with poor sanitation and polluted water is characteristics of the disease picture in many of the developing countries of the world. Thus, rural people of developing world need local water treatment technological which should be reliable, rapid, simple and convenient. The development and introduction of appropriate technological are of prime concern if the developing countries are ever to share the benefits of improved water quality.

6.2 CONCLUSION

Hence, during the study period different water samples from drinking water source and river were collected and processed for chemical and microbiological quality. From the study it was found that all water samples were microbiologically unsafe and need to be treated.

Chapter-VII

7. SUMMARY AND RECOMMENDATION

7.1 Summary

Water samples from different natural water sources such of Sudhanghat River, canal etc. were investigated bacteriologicallly in Kathmandu city. None of water samples from different sources were found bacteriologically safe in this study. All the water samples contained higher number of coliform bacteria like *Escherichia coli* and *Klebsiella sps* were isolated from some water sources.

In this study, physico-chemical parameters of water samples also analysed, several parameters were tested to determine the physico- chemical quality of water and most were within the WHO standards for drinking water (1993). Chemically water samples of natural sources were safe for drinking purpose.

7.2 Recommendation

1. Great efforts should be taken to improve sanitary conditions and hygienic environment of natural water sources in communities especially in rural areas.
2. Government and local people should play greater attention to the presentation and maintenance of water sources in rural communities.
3. Government and responsible organization should provide education regarding sanitary practices and maintenance of hygienic environment of rural community. The improvement of community water supply and community health education should be politically committed at National level.

4. Active participation of rural people and woman's involvement it especially important for development prevention and control of water pollution in communities.
5. A regular water quality monitoring system should be provided to check out the causes of water pollution of water sources in rural communities.
6. Simple water treatment Methods should be analyzed and monitored for rural communities.
7. Government and water authorities should set a national standard relating to the quality of water.

CHAPTER-VIII

8. REFERENCES

ADB (1985): Nepal water supply and sanitation sector profile, Asian Development Bank, Manila.

Adhikari S. and Sharma, A.P(1987): Anti Microbial Activities of some plant Extracts, Journal of Natural history Museum, 11(1-4),Nepal.

Aaejd, K.R.(1993): Experiments in Microbiology, Plant Pathology and tissue culture, wishwas prakashan.

APHA (1989): Standard Methods for the Examination of water and waste water. American Public Health Association, Washington DC, 17th edition, 1995.

Benson H.J (1979): Microbial Applications A Laboratory Manual in General Microbiology, 3rd Edition. Web. Wm.C . Brown company Publishers Dubuque, Iowa.

Bergey's Manual of systematic Bacteriology Vol. (1986) : Sneath PHA, Mair NS, Sharpe ME, Holt J.G, Williams and Wilkins, Baltimore , London, Los Angeles, Sydney, Tokyo.

Bhatta, D.D. (1970): In Natural History of Economic Botany of Nepal First edition, Department of Information HMG's of Nepal.

CBS (1989): Statistical Year Book of Nepal 1989. HMG/NPC Central Bureau of Statistics, Kathmandu.

CBS (1991): Statistical Year Book of Nepal, 1991 : HMG/NPC Central Bureau of Statistics : 1991:PP.211-215.

CEDA(1989): A study on the Environmental Problems due to urbanization in some selected Nagar Panchayats of Nepal. Report Submitted to UNDP/Kathmandu. Centre for economic development and Administration, Tribhuvan University, Kathmandu.

Chesbrough M. (1984): Medical Laboratory Manual for Tropical Countries. Volume II: Microbiology, ELBS Edition, Tropical Health Technology, Butter Worth – Heinemann ltd.Linacre House, Jordan Hill, Oxford OX 2 8 DP.

Chopra:I.C.; Chopra, S.L.; Nayar, R.N. (1950): In glossary of Indian Medicinal Plants, New Delhi.

Chopra:I.C.; Chopra, R.N. (1955): A Review of work on Indian Medicinal Plants, Special Report Series No.30, Indian Council of Medical Research, New Delhi.

Clark J.W.; Viessman, W.; Hammer, M.J. (1977): Water supply and Pollution Control, Third Edition IEP-Dun Dunnelley, Harper and Row, publisher, New York.

DISVI (1988):Pollution Monitoring of the Bagmati River. Preliminary Report, DISVI, Kathmandu.

DISVI (1989): Water Quality Monitoring of Kathmandu City Water Supply. Final Report. Health Education and Service Project DISVI, Kathmandu.

DISVI(1990c):Water Quality Assessment in Terai Tubewell Project. Health Education and Service Project, DISVI, Kathmandu.

DISVI(1990 b):Water Quality Testing in Ilam. DISVI, Kathmandu.

ENPHO/ DISVI (1990) : Water quality of stone taps (Kathmandu city) .Enviroment and public health Organization and ,DISVI, Kathmandu.

FAO (1992) : Manual of food quality control, 4Rev-1, Microbiological Analysis,FAO, Rome.

HMG/Nepal (1980) : International Drinking Water Supply and Sanitation Decade:Community water, supply and sanitation country report, DWSS, Kathmandu 4 May, 1980

HMG/WHO (1987) : Basic Information Indicators to support implementation of Basic Minimum Needs and HFA/2000 strategies,Nepal

Hugo, W.B; Rusell, A.D. : Pharmaceutical Microbiology, first edition, 1997

IUCN (1990) : Environment pollution in Nepal. A review of studies .HMG/NPC in Collaboration with IUCN. The world conservation union.

IWTC (1990) : Woman and water. International Women's Tribune Centre (IWTC)777 United Nations Plaza, New York, NY 10017, July 1990.

John, S.A.A(1986) : Proper use of African natural coagulants for rural water supplies. GTZ, Eschborn, Germany.

Joshi, A.R. (1987): A study of environmental relationship of certain village communities in the Central Development Region of Nepal, Ph.D. Thesis, University of wales, Cardiff, U.K.

Mackie and McCartney (1989): Practical Medical Microbiology, 13th edition Churchill Livingstone, Melbourne and New York.

Manja, et.al. (1982): A simple field test for the detection of faecal pollution in drinking water. Bull. WHO, 10:797-801.

Meybeck, et.al. (1990): Global Environment Monitoring system, Global fresh water Quality a first assessment, pp 42, 288 published on behalf of the WHO and UNEP by Black Well Reference.

Michael, J.P. Reid, R.D. and Chan, E.C.S. (1970): Microbiology Tata McGraw- Hill Publishing Company, India.

Mifsud, F.M. (1986) : International Ground Water Resources Law. "Foreword" in J.A Barberis. FAO Legislative Study 40, FAO Rome.

Ministry of Health/ WHO Nepal (1977): Screening of some medicinal plants for antifungal activity Geobios 4,129

NCS (1994): Nepal Chemical society, proceedings of International seminar on water and Environment (30 March-1 April, 1994, Kathmandu).

Pant,A.P (1994): Prevention and Control of water pollution: A comparative legal study of India and Nepal with special reference to their common environmental problems, Dissertation (Ph.D. in Law), University of Delhi.

Ranjitkar, A.K. (1985): Water Supply and Treatment Technology in Developing Countries – Application to Nepal. M. $4.00X10^{6}$Sc. Thesis, Mississippi State University, Mississippi, May-1985.

Ronast (1988b): Water Quality of Bagmati River in the Pashupati Area. Royal Nepal Academy of Science and Technology (RONAST), Kathmandu.

RONAST (1988 a): Pollution Monitoring of the Bagmati River. Royal Nepal Academy of Science Technology (RONAST) Kathmandu.

Shakya, P.M. (1982): Preliminary studies on some plants for their Antimicrobial activities , proceeding of First National Science and Technology Congress , Kirtipur, Kathmandu: pp.136.

Sharma, A.P. (1987): Status of Pollution in Nepal. Readings on Environmental Issue. MAB Bulletin No. 8 Nepal National Committee For Man and the Biosphere, Kathmandu, pp. 53-61.

Sharma, A.P. (1986): Kathmandu Drinking Water Monitoring. Report Submitted to Nepal National Committee for Man and the Biosphere, Kathmandu.

Sharma, A.P. (1978): The Quality of Drinking Water in Kathmandu J. Inst. Sc.: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 Nepal, 1993.

Sharma, A.P. and Rijal, A. (1988): Impact of Industrial Effluent on Pollution of River and Adjoining Land. Report Submitted to Nepal National Committee for Man and the Biosphere, Kathmandu.

Sharma, A.P. (1993): Status of Sewerage and Industrial Effluent of Nepal, Environments Jr. 3, No. 1, 1993.

Shrestha, R and Sharma A.P. (1988): Antimicrobial activity of some Essential oil, First Regional Conference of Association of plant physiologist of SAARC countries (APPSC) on the role of plant physiology and Biotechnology in plant productivity.

Sutherland, J.P. Folkard, G.K, Grant, W.D (1990). Natural Coagulants for appropriate water Treatment a novel approach waterlines, 8, 4 April, 30-32.

The Wealth of India (1976): A Dictionary of Indian Raw Materials and Industrial Products, Publications and Information Directorate, CSIR, Hillside Road, New Delhi-10012, First Publication.

Tricedi, R.K. Goel, P.K. (1986): Chemical and Biological Methods for water pollution studies. Environmental Publication, Post Box, Karad 415110.

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

Vaidya, S. and Karma Charya, S. (1986): A study of Ground and surface water of Kathmandu Valley. NCST Newsletter, 5(1): 6-7.

WHO (1986): Establishing and Equipping Water Laboratories in Developing Countries. World Health Organization, Geneva, 1986.

WHO (1982): Global Strategy for Health for all by the year 2000, WHO, Geneva, Switzerland.

WHO (1984): Guidelines for Drinking water Quality, Volume I World Health Organization, Geneva, 1984.

WHO (1984): Guidelines for Drinking water Quality, Volume III World Health Organization, Geneva, 1984.

WHO (1993): Guidelines for Drinking water quality, Volume I World Health Organization, Geneva, 1993.

WHO (1993): Guidelines for Drinking water quality, Volume II World Health Organization, Geneva, 1993.

WHO (1993): Guidelines for Drinking water quality, Volume III World Health Organization, Geneva, 1993. .

APPENDIX-1

Composition and preparation of Media used in Isolation and Identification of Bacteria.

(i) CULTURE MEDIA

1. Nutrient Agar

Composition	(Gram/Litre)
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15.0
Final PH (at 25° C)	7.4 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes.

2. Nutrient Broth

Composition	(Gram/Litre)
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final PH (at 25° C)	7.4 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes.

3. Plate Count Agar

Composition	(Gram/Litre)
Tryptone	5.0
Yeast Extract	2.5
Dextrose	1.0
Agar	9.0
Final PH (at 25° C)	7.4 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes.

4. Lactose Broth

Composition	(Gram/Litre)
Beef Extract	3.0
Pancreatic digest of galatin	5.0
Lactose	5.0
Final PH (at 25° C)	6.9 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes

5. Mac-Conkey Agar

Composition	(Gram/Litre)
Peptone	20.0
Lactose	20.0
Bile salts	5.0
Sodium Chloride	5.0
Neutral Red	0.075
Agar	12.0
Final PH (at 25° C)	7.4 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes.

6. Voilet Red Bile Agar

Composition	(Gram/Litre)
Yeast Extract	3.0
Peptone	7.0
Bile Salt No. 3	1.5
Lactose	10.0
Sodium Chloride	5.0
Agar	15.0
Neutral Red	0.03
Crystal Violet	0.002
Final PH (at 25° C)	7.4 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121°c) for 15 Minutes.

7. Seilenite-F-Broth

Composition	(Gram/Litre)
Tryptone	5.0
Lactose	4.0
Sodium Phosphate	10.0
Sodium Acid Selenite	4.0
Final pH (at 25^{o}C)	7.4+0.2

Warmed to dissolve the medium and mixed well then sterilized in a boiling water bath for 10 minutes. The media was not autoclaved and excessive heating was avoided.

8 Salmonella-Shigella (S-S) Agar

Composition	(Gram/Litre)
Beef extract	5.0
Peptone	5.0
Lactose	10.0
Bile Salts	8.5
Sodium Citrate	10.0
Sodim tiosulphate	8.5
Ferric Citrate	1.0
Brilliant green	0.00033
Neutral Red	0.025
Agar	15.0
Final pH (at 25^{o}C)	7.0 ± 0.2

Heated to boil with frequent agitation to dissolve the medium completely. The medium was not autoclaved and over heating was avoided

9 M-Endo Agar

Composition	(Gram/Litre)
Yeast extract	6.0
Peptone	20.6
Lactose	25.0
Dipotassium phosphate	7.0
Basic Fuchsin	1.0
Sodium Sulphite	2.5
Agar	15.0
Final pH (at 25^{o}C)	6.9 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

10 Brilliant Green Agar

Composition	(gm/Litre)
Protease peptone	10.0
Lactose	10.0
Yeast extract	3.0
Sodium Chloride	5.0
Sucrose	10.0
Phenol Red	0.08
Brilliant Green	0.0125
Agar	20.0
Final pH (at 25ºC)	6.9 ± 0.2

Sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

APPENDIX – 2

COMPOSITON OF STAINS AND REAGENTS

1 Gram's Staining

Heat fixed smear of bacterial culture was flooded with crystal violet for one minute and excess stain was washed out. The slide was treated with Gram's Iodine for 1 minute and washed. It was flooded with 95% emul alcohol immediately and washed with water. Then smear was treated with safranin for 1 minute and washed with water. It was dried and observed under microscope.

Preparation of Stains

i. Crystal Violet

Solution A

Crystal Violet	2.0gm
95% ethyl alcohol	20.0ml

Solution B

Ammonium oxalate	0.8 gm
Distilled water	30.0 gm

Crystal violet was dissolved in ethyl alcohol and ammonium oxalate in distilled water. Then solution A and B were mixed.

ii. Gram's Iodine

Iodine	1.0 gm
Potassium Iodine	2.0 ml
Distilled water	300.0 ml

Iodine and potassium Iodine were dissolved in distilled water.

iii. Ethyl Alcohol (95%)

Absolute alcohol	95.0 ml
Distilled water	5.0 ml

iv. Safranin

Safranin (2.5% solution in 95% ethyl alcohol)	10.0 ml
Distilled water	100.0 ml

2 Reagents for Do and BOD Calculation

Preparation of Reagents

1. Alkaline Potassium Iodide Solution

70gm potassium hydroxide (KOH) and 15 gm of potassium iodine (KI) was placed into a conical flask and total volume was made upto 100 ml by adding distilled water.

2. Manganous Sulphate Solution ($MnSO_4$)

i. Required weight of manganous sulphate was taken in a flask.

$MnSO_4$. H_2O ……364 gm
$MnSO_4$. $2H_2O$……400 gm
$MnSO_4.4H_2O$……480 gm
$MnSO_4.5H_2O$……500 gm

ii. Total volume was made 100 ml by adding hot and distilled water.

iii. This solution filtered by using Whatman No. 1 filter paper.

iv. The filtered solution was kept in brown bottle.

3. Preparation of 0.025 N sodium thiosulphate solution ($Ma_2S_2O_3$).

for 1.000N, sodium thiosulphate 243.17 gm is dissolved in 1000ml
for 0.025N '' 248.17 x 0.025 gm '' ''
0.025 N '' $\frac{248.17 \times 0.025 \text{ gm}}{5}$ '' ''
= 1.34 gm '' ''

Hence, 1.34 gm sodium thiosulphate was placed in flask and total volume of solution was made 200 ml after adding distilled water.

4. Preparation of 0.5% starch solution

0.05 gm of starch was placed into flask and total volume was made 1 ml and solution was boiled and cooled.

APPENDIX – 3

Composition of phosphate Buffer (Mackie and McCartney, 1989)

Stock solution A: 0.2 mol/litre solution of monobasic sodium phosphate (31.2 g NaH_2PO_4. $2H_2O$ in 1000 ml)

Stock solution B : 0.2 mol/litre solution of diabasic, sodium Phosphate (28.39 g of Na_2HPO_4 or 71.7 gm of Na_2HPO_4. $12H_20$ in 1000ml)

X ml of solution A and Y ml of solution B was mixed and diluted to 200 ml to obtain 0.2 m buffer of required pH.

Composition of phosphate buffer

X ml of A	Yml of B	pH
92.0	8.0	5.8
87.7	**12.3**	**6.0**
81.5	18.5	6.2
73.5	**26.5**	**6.4**
62.5	37.5	6.6
51.0	**49.0**	**6.8**
39.0	61.0	7.0
28.0	**72.0**	**7.2**
19.0	81.0	7.4
13.0	**87.0**	**7.6**
8.5	91.5	7.8
5.3	94.7	8.0

Photo1: Petri plate showing *Salmonella sps* on S-S agar

Photo 2: Biochemical test of *Salmonella sps*

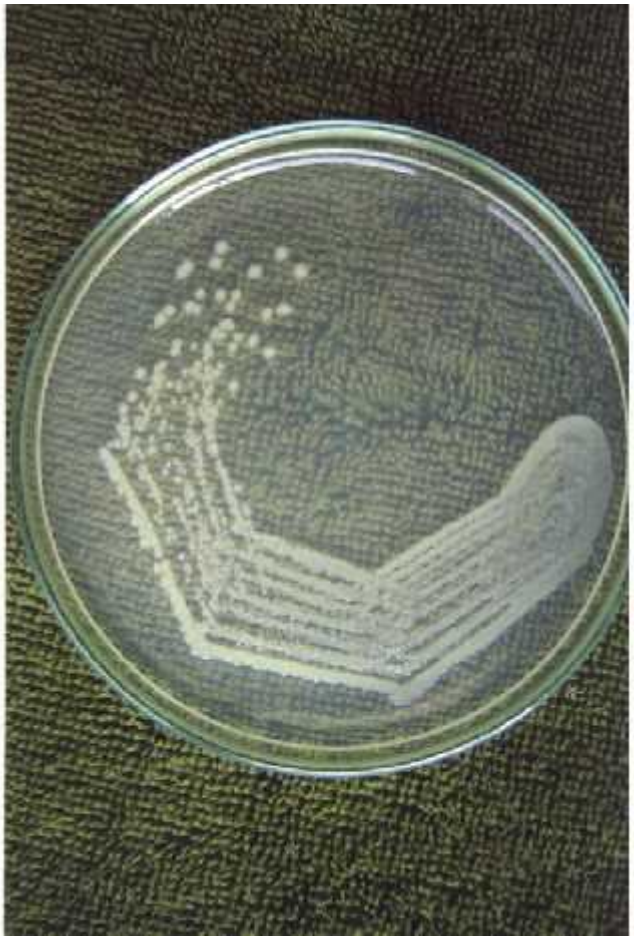


Photo 3: Petri plate showing *E-coli* on Nutrient agar

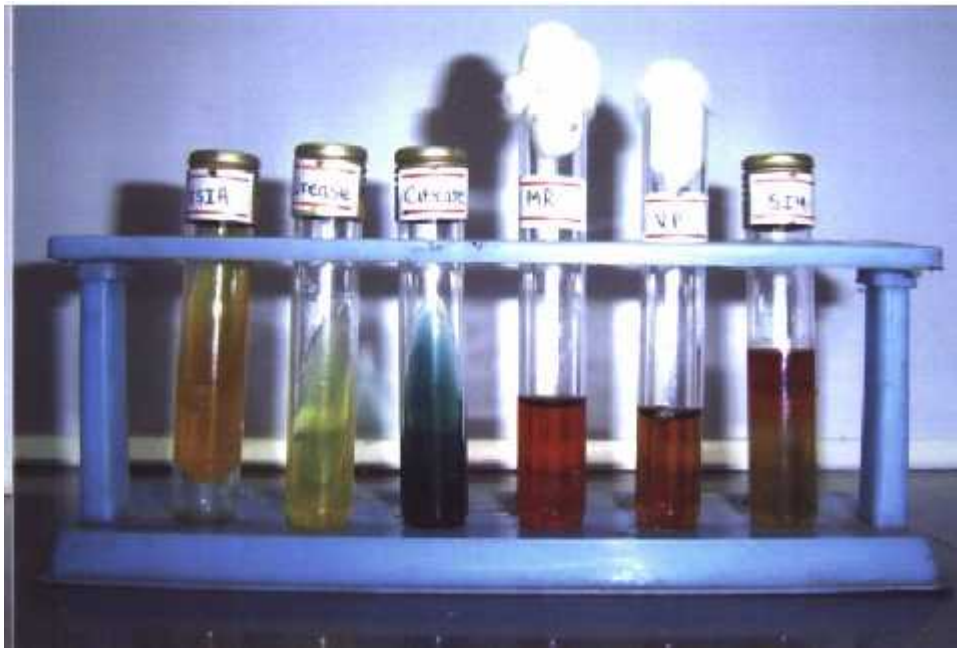
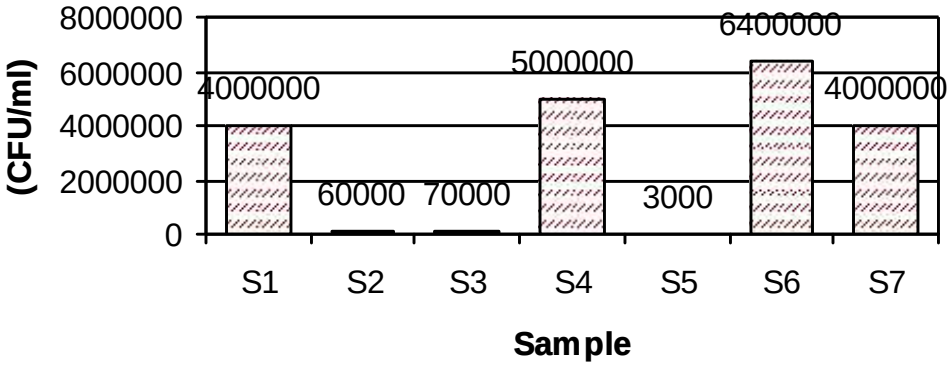
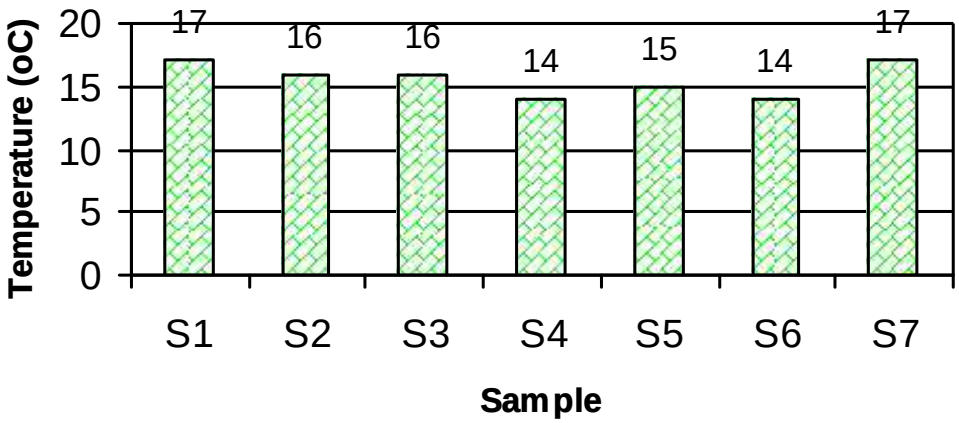


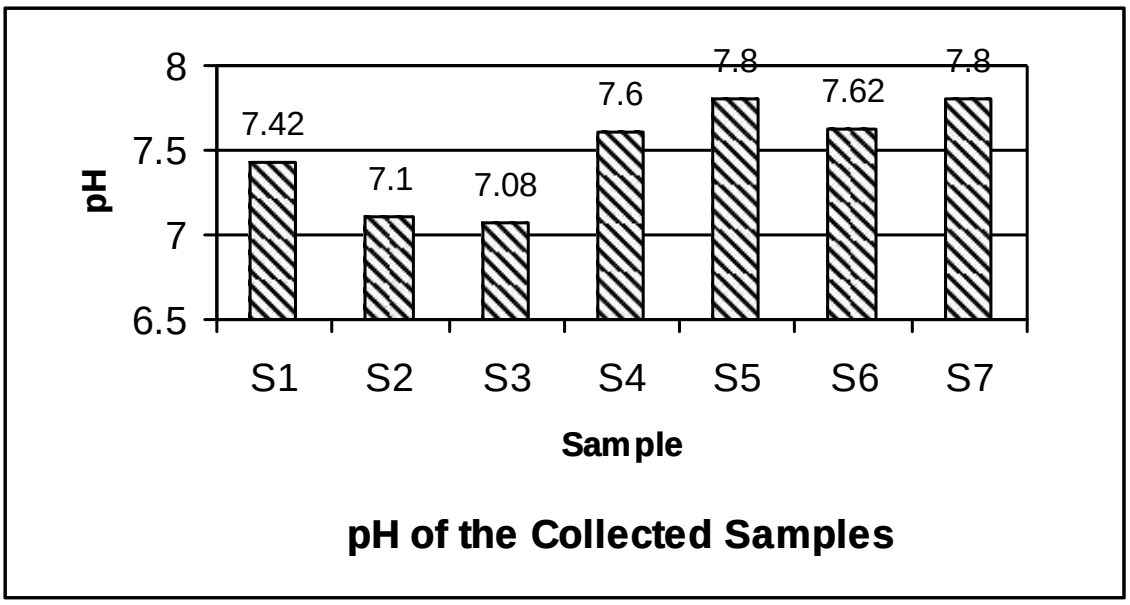
Photo 4: Biochemical tests of *E-coli*



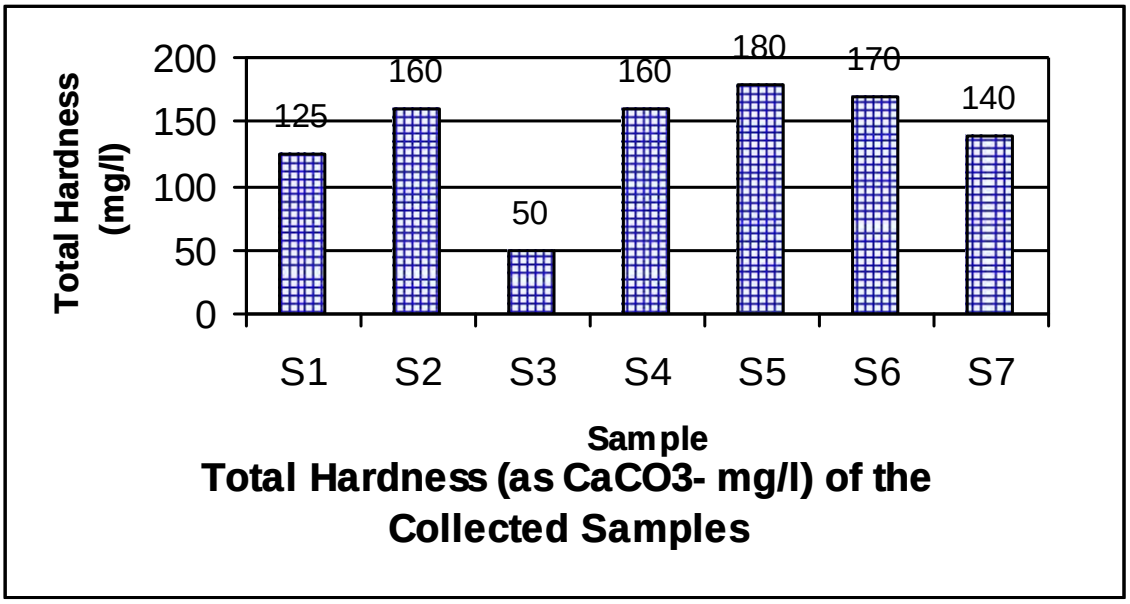
Total Bacterial Count of the Collected Samples



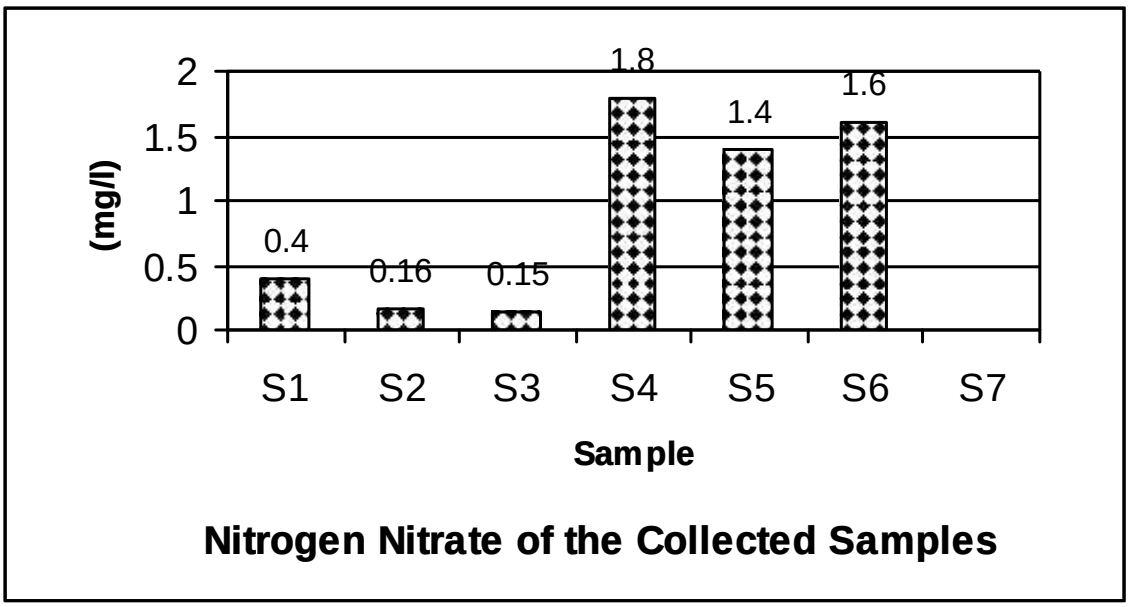
Temperature of the Collected Samples



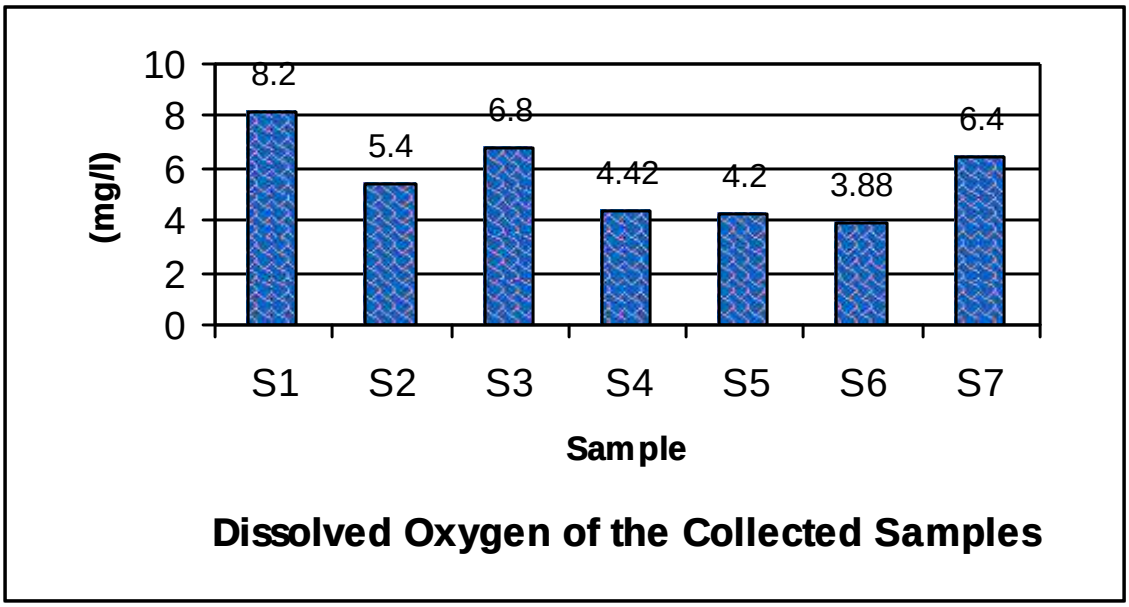
pH of the Collected Samples



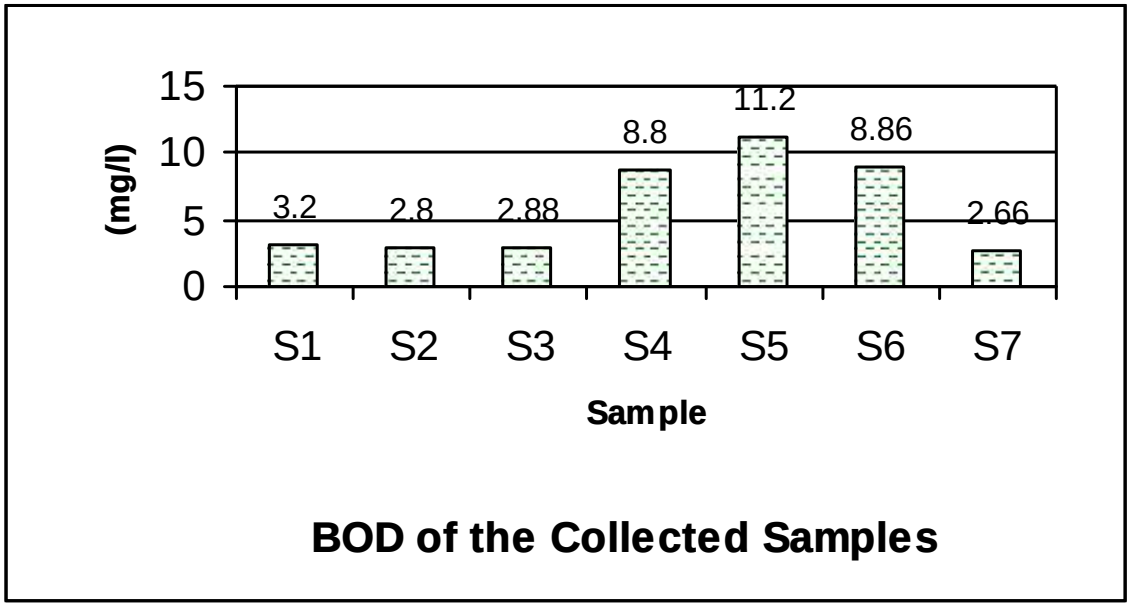
Total Hardness (as CaCO3- mg/l) of the Collected Samples



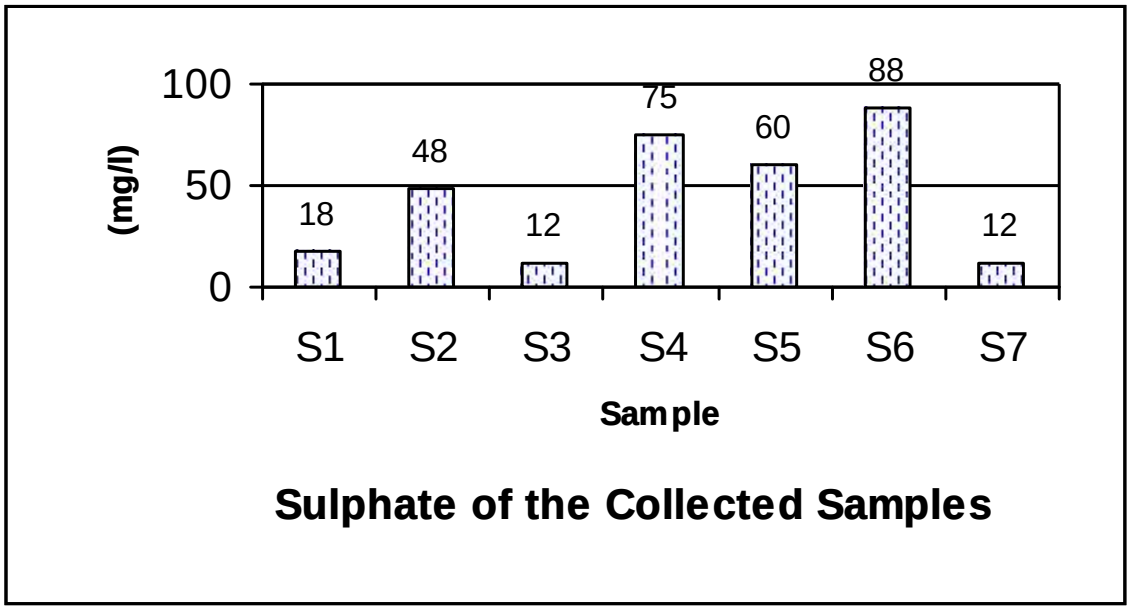
Nitrogen Nitrate of the Collected Samples



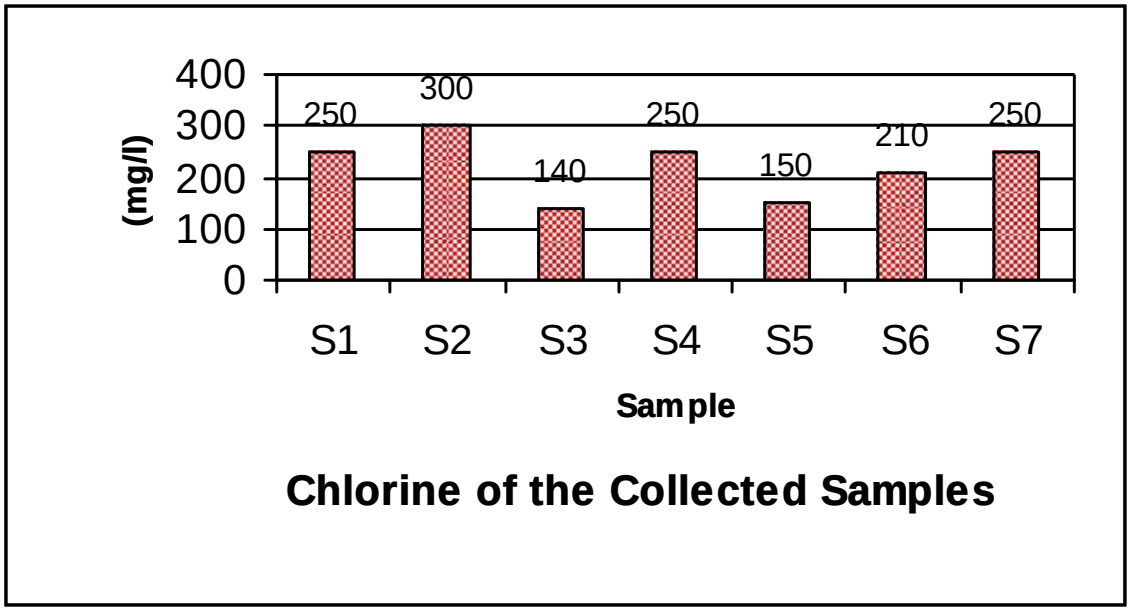
Dissolved Oxygen of the Collected Samples



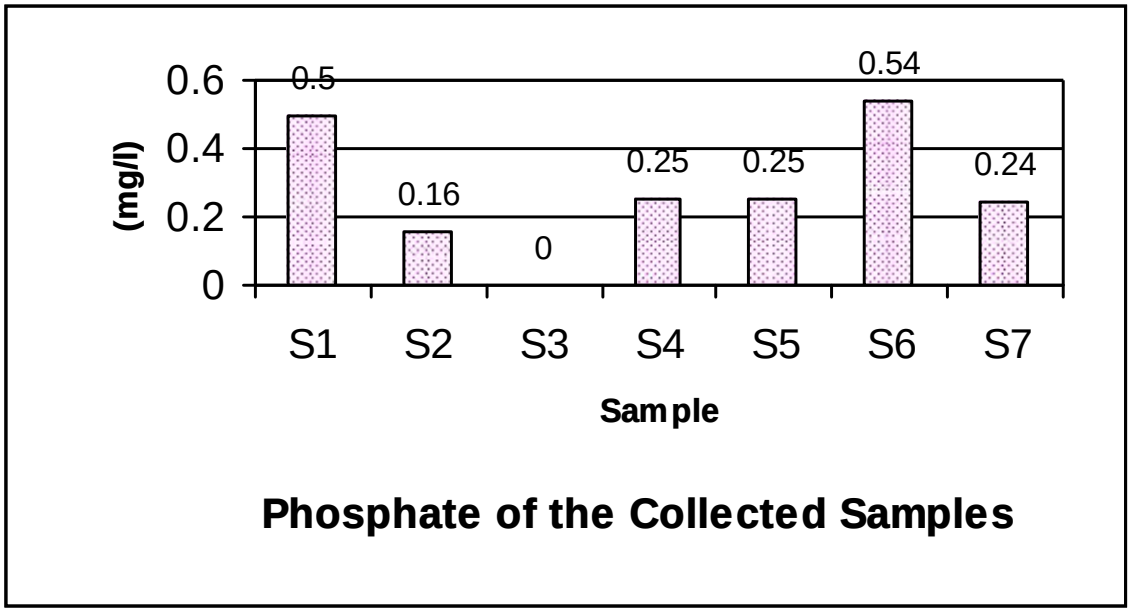
BOD of the Collected Samples



Sulphate of the Collected Samples



Chlorine of the Collected Samples



Phosphate of the Collected Samples