ASSESSMENT OF GROUNDWATER QUALITY, AND STUDY OF ANTIBIOTIC RESISTANCE AND OLIGODYNAMIC ACTION AGAINST SOME ISOLATED ENTERIC BACTERIA

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ASSESSMENT OF GROUNDWATER QUALITY, AND STUDY OF ANTIBIOTIC RESISTANCE AND OLIGODYNAMIC ACTION AGAINST SOME ISOLATED ENTERIC BACTERIA

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RECOMMENDATION

This is to certify that ***Mr. Makhan Maharjan*** has completed his dissertation work entitled **"ASSESSMENT OF GROUNDWATER QUALITY, AND STUDY OF ANTIBIOTIC RESISTANCE AND OLIGODYNAMIC ACTION AGAINST SOME ISOLATED ENTERIC BACTERIA"** as a partial fulfilment of the M.Sc. Degree in Microbiology under my supervision. To my knowledge his work has not been submitted for any other degree.

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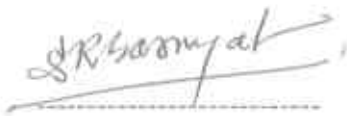

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ABSTRACT

A total of 70 water samples randomly collected from 5 shallow pumps, 3 shallow wells, 14 stone spouts and 48 dug wells in urban area of Patan city (Nepal) were analyzed for physical, chemical and bacteriological quality. Out of these, 85.6% of samples showed presence of total coliforms and 68.6% contained faecal coliforms. However, 68.6% exceeded the WHO permissible level for drinking water. Sanitary indicator bacteria were detected from all the supplies except shallow pumps. In this study, 120 enteric bacteria were isolated from 49 samples. Recovery of *Enterobacter sp.* was maximum followed by *Escherichia sp.* > *Citrobacter sp.* > *Salmonella sp.* and others.

At the same time physico-chemical parameters were also analyzed. Most of water samples showed values within the WHO standard limit for drinking water. The values for turbidity, conductivity, hardness, iron, ammonia and chloride were found above maximum permissible levels for 12.9%, 5.7%, 1.4%, 12.9%, 38.6% and 1.4% of samples, respectively.

Isolated enteric bacteria were also assayed for antibiotic resistant patterns, and 82.5% of the enterobacteria were resistant to at least one antibiotic, with resistance most commonly directd toward nitrofurantoin (68.3%), ampicillin (44.2%) and tetracycline (28.3%). Frequency of multiple-antibiotic resistance (MAR) against ten common antibiotics within each species was also determined. During this study, 54.2% of the total isolates were MAR, including 23.8% , 28.6% , 33.3% , 33.3 % and 100% of *Escherichia coli*, *Salmonella sp.*, *S. paratyphi A* , *Shigella dysenteriae* and *Klebsiella sp.*, respectively.

The toxic effect of heavy metals upon bacteria (oligodynamic action) was studied against some enteric bacteria isolates. Silver and copper were found to be very effective, while comparatively brass less effective, and aluminium and steel were found to be ineffective for their lethal effect against tested organisms.

During the study it was found that 48.6% water sources were good from sanitary points of view and others (51.4%) were in poor condition. Almost all water sources (87.1%) were extensively used by the local people for drinking. Most of the people (60%) suffer with waterborne diseases several times in the year.

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LIST OF ABBREVIATIONS

Abbreviation		Referent
ADB	-	Asian Development Bank
APHA	-	American Public Health association
BOD	-	biochemical oxygen demand
°C	-	degree (s) Celsius
CBS	-	Central Bureau of Statistics
CDHP	-	Community Development through Health Programme
CEDA	-	Centre for Economic Development and Administration
CFU	-	colony forming unit
cm	-	centimetre (s)
col	-	coliforms
DISVI	-	Italian International Co-operation
DO	-	dissolved oxygen
ENPHO	-	Environment and Public Health Organization
GTZ	-	Deutsche Gesellschaft fur Technische Zusammenarbeit (German Technical Co-operation)
HMG	-	His Majesty's Government
hrs	-	hours
INGO	-	International Non-Governmental Organization
MAB	-	Man and Biosphere Committee
MF	-	Membrane Filter
ml	-	millilitre
MID	-	million litre per day
µS	-	micro Siemens
NGO	-	Non-Governmental Organization
NTU	-	nephelometric turbidity unit (s)
NWSC	-	Nepal Water Supply Corporation
RONAST	-	Royal Nepal Academy of Science and Technology
SWMRMC	-	Solid Waste Management and Resource Mobilisation Centre
UDLE	-	urban development through local efforts
UMN	-	United Mission to Nepal
UNICEF	-	United Nations Children's Fund
VDC	-	Village Development Committee

CHAPTER - ONE

INTRODUCTION

Clean, protected and safe water is an absolute need for healthy, productive life. There is no denying the vital importance of water and there is no substitute for it as well. However, water can also be the carrier of suffering and the cause of health hazard.

Safe drinking water is defined as water free from micro-organisms and chemicals in concentrations which could cause acute or long-term adverse effects on human health. It must also be fairly clear, palatable, odourless, and not brackish (salty), and must not stain clothes washed in it or affect the taste of food cooked in it (Srivastava, 1990). In November 1980, the General Assembly of the United Nations formally declared 1981-1990 as the International Drinking Water Supply and Sanitation Decade (IDWSSD) with the goal of providing safe drinking water and adequate sanitary facilities for all. in an endeavour to protect public health.

Water fit for drinking exists in the ground in some form at some depth nearly everywhere on earth. Groundwater is generally better and safe as a source of drinking water than that of surface water due to the soil filtering mechanism. Traditionally, man has always extracted water from the ground through hand-dug wells. This method is supremely successful and has been used throughout the world for thousands of years. Groundwater is very important source of water, and in some areas it is the only source of water . Increasing demand for potable water has forced the growing use of groundwater throughout the world.

Water pollution, today, is one of the most serious environmental issues all over the world. Pollution of water is responsible for a very large number of mortalities and incapacitations in the world. Thousands of people die or suffer from water and sanitation related diseases. Water, therefore, the most vital resource for all kinds of life on this planet, can be extremely dangerous when it becomes the vehicle of transmission of disease. Epidemic waterborne diseases cause emotional and economic losses in communities affected.

Human activities create vast amounts of various wastes and pollutants which led to the deterioration of quality of water resources. Rapid population growth due to massive migrations to the urban areas, unsystematic and uncontrolled urbanization and industrialisation, lack of proper sewage disposal system, utilization of excess fertilizers and pesticides in agricultural land, traditional habit of using open fields and stream sides as open latrine etc. are some causes responsible for water pollution through surface run-off and infiltration processes.

Site of Study : Patan, one of the historical cities of the Kathmandu Valley (Nepal), is situated five kilometres south-east of Kathmandu across the southern bank of the river Bagmati. The city has many stone spouts and hundreds of dug-wells still functioning and serving present day water needs of the thousands of local people. The stone spouts are beautifully carved out of stone in many different architectural styles and were recorded to be built at different periods-in between the tenth and the seventeenth century during the Licchhavi and the Mall periods (Sanday, 1982). These ancient spouts incorportate sand and charcoal filters (Pradhan *et al.*, 1972). Similarly, wells have been dug since ancient times. The traditional water supply system is a significant alternative for the people of Patan since inadequate water is provided by municipal water supply.

The emergence of antibiotic resistant (AR) bacteria has become a growing public health concern in recent years. The presence and persistence of AR bacteria, particularly multiple-antibiotic resistant (MAR) bacteria is a serious threat to mankind. It is making difficult to treat some infectious diseases which can contribute to the spread of major infectious diseases causing serious epidemics. Several studies have been conducted on resistance of bacteria to antimicrobial agents in other countries.

Extremely small amounts of certain heavy metals can exert a lethal effect upon bacteria. From ancient time metallic water pots have been in common use in most parts of Nepal.

OBJECTIVES OF THE STUDY

Groundwater supplies are important and suitable sources of drinking water. Hence, their protection and conservation are of utmost importance.

General Objective

* To assess the groundwater quality, and to study antibiotic resistance and oligodynamic action against some common enterobacteria isolated.

Specific Objectives

* To assess the microbial quality of groundwater.
* To study the physico-chemical parameters of groundwater.
* To isolate some common enteric bacteria from groundwater.
* To study the antibiotic resistance pattern against enteric bacteria isolates of groundwater.
* To study oligodynamic action against some of the isolated enterobacteria.
* To provide baseline data for future use.

CHAPTER - TWO

LITERATURE REVIEW

2.1 Water Pollution

Water pollution is any physical, chemical or biological change in surface water or groundwater that can adversely affect living organisms. (Water pollution is one of the most important problems being faced by both developed and developing world together.) Pollution of water is responsible for a very large number of mortalities and incapacitations in the world. Availability of clean water is going to become the greatest constraint for development tomorrow. Groundwater pollution is much more difficult to detect and control than surface water pollution. Any pollution of water resources is potentially a hazard to human health, destructive to aquatic life, aesthetically deplorable, a future economic burden to society, and damaging to entire life systems in the natural environment (Miller, 1988 and Trivedy and Goel, 1986).

2.2 Groundwater

2.2.1 Groundwater - An Increasing Demand

"*Underground Water, An Unseen Source*," the slogan for World Drinking Water Day (WDWD) for 1998 well explains the importance of groundwater in the present context. Groundwater constitute 1.7 percent (or 23.4 million km^3) of the total 1.384 billion km^3 water on Earth (Mays, 1996). Depending on the soil filtering mechanism, groundwater is usually considered as clean and free from pathogenic organisms. Water fit for drinking exists in the ground in some form at some depth nearly everywhere on earth. Groundwater serves as a very important source of water in most areas of the world, more so in the areas where surface water resources are scarce (Morgan, 1990).

Traditionally, man has always extracted water from the ground through dug wells, tube wells etc. This method is supremely successful and has been used throughout the world for thousands of years. It is estimated that in Zimbabwe alone nearly 100,000 wells or water holes may be in operation daily for most of the year, and it is certain that more water is gathered from this source compared with any other in the rural areas of Zimbabwe (Morgan, 1990). Groundwater is a vital resource that provides drinking water for one out of two Americans and 95% of those in rural areas. About 75% of public water supplies in the United States use groundwater as a raw water source, and nearly 100% of private, domestic water supplies rely on groundwater as a

drinking water source (Miller, 1988; Allen and Geldreich, 1975). A recent U.S. Geological Survey study indicated that more than 625,000 West Virginians use private wells or springs as their source of drinking water (Suder and Lessing 1985, quoted by Bissonnette *et al.* 1987). Over the 35 years 1935-1970, the use of groundwater in the United States increased on a yearly basis from 28 km^3 to 90 km^3. In England and Wales, approximately 30% of water demand is met through the use of groundwater. In arid regions, such as Israel and Southern California, groundwater can account for up to 70% and 50%, respectively, of the total water supply. The amount of groundwater withdraw from wells at Cairo Water works ranges from 0.4 to 0.6 million m^3 day^{-1} representing about 11-17% of the total amount of water consumed in Cairo (Hosny, 1990).

In Nepal as well, the shortage of drinking water has become a serious problem. The people of Kathmandu have become used to living with dry taps. Householders were forced to go back to their old wells and water spouts. The piped water supply has never been sufficient to meet even the minimum demand of the people. The traditional water supply system from stone spouts and dug wells is a significant alternative for the people of Patan. The dug wells are a key-water resource largely overlooked until now (Joshi, 1993).

This dramatic rise in groundwater use has created several concerns for the protection of the quality and quantity of this source.

2.2.2 Mechanisms for Groundwater Pollution

Pollution of the environment results from the wide range of human activities. Groundwater can be contaminated through a variety of mechanisms by numerous contaminants from a number of point and non-point sources. Groundwater can be contaminated by localized releases from sources such as hazardous waste disposal sites, municipal landfills, surface impoundments, underground storage tanks, gas and oil pipelines, back-siphoning of agricultural chemicals into wells. Groundwater can also become contaminated by substances released at or near the soil surface in a more dispersed manner, including pesticides, fertlizers, septic tank leachate, and contamination from other non-point sources (Mays,1996). The traditional habit of people to use open fields and stream sides as latrines and the ignorance on their part to see the dangers following their action equally contribute to the water pollution (Pradhan *et al.*, 1972).

2.3.1.1 ***Escherichia coli* and the coliform bacteria**

Escherichia coli

E. coli is a member of the family Enterobacteriaceae, and is characterised by possession of the enzymes β-galactosidase and β-glucuronidase. It grows at 44-45°C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. Some strains can grow at 37°C, but not at 44-45°C, and some do not produce gas. *E. coli* does not produce oxidase or hydrolyse urea. It is usually motile, and gives a positive methyl-red reaction and a negative Voges-Proskauer reaction, and does not utilize citrate, grow in KCN or liquefy gelatin (WHO, 1993 and Collee *et al.*, 1989).

Thermotolerant coliform bacteria

These are defined as the group of coliform organisms that are able to ferment lactose at 44-45°C; they comprise the genus *Escherichia* and, to a lesser extent species of *Klebsiella, Enterobacter,* and *Citrobacter*. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. The concentrations of thermotolerant coliforms are, under most circumstances, directly related to that of *E. coli*. Hence, their use in assessing water quality is considered acceptable for routine purposes (WHO, 1993).

Coliform organisms (total coliforms)

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality largely because they are easy to detect and enumerate in water. The term "*coliform organisms*" refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35-37°C with the production of acid, gas, and aldehyde within 24-48 hours. They are also oxidase-negative and non-spore-forming, and they display β-galactosidase activity. Coliform bacteria belong to the genera *Escherichia, Citrobacter, Enterobacter and Klebsiella* (WHO, 1993).

2.3.1.2**Faecal streptococci**

The term "faecal streptococci" refers to those streptococci generally present in the faeces of humans and animals. All possess the Lancefield group D antigen. Taxonomically, they belong to the genera *Enterococcus* and *Streptococcus*. The genus *Enterococcus* includes several

species, and in the genus *Streptococcus*, only *S. bovis* and *S. equinus* possess the group D antigen and are members of the faecal streptococcus group. Faecal streptococci rarely multiply in polluted water, and they are more persistent than *E. coli* and coliform bacteria. Their primary value in water quality examination is therefore as additional indicators of treatment efficiency (WHO, 1993).

2.3.1.3 **Sulfite-reducing clostridia**

These are anaerobic, spore-forming organisms, of which the most characteristic, *Clostridium perfringens* (*C. welchii*), is normally present in faeces, although in much smaller numbers than *E. coli*. However, they are not exclusively of faecal origin and can be derived from other environmental sources. Clostridial spores can survive in water much longer than organisms of the coliform group and will resist disinfection. Their presence in disinfected waters may thus indicate deficiencies in treatment, and deficiencies in filtration practice in filtered supplies (WHO, 1993).

2.3.1.4 **The use of *E. coli* as an Indicator Organism**

E. coli is present in the intestine of warm-blooded animals, including humans. Therefore, the presence of *E.coli* in water samples indicates the presence of faecal matter and of the possible presence of pathogenic organisms of human origin. *E. coli* has been used and continues to be considered a good indicator organism for the following reasons :

- *E. coli* are found in large numbers in the faeces of humans and warm-blooded animals (10^9 per gram in fresh faeces).
- *E. coli* are relatively easy to detect and assay.
- *E. coli* responds the same as pathogens to changes in the aquatic environment.

The most specific of the readily detectable faecal indicator bacteria and the one present in greatest numbers in faeces is *E. coli* and it is therefore recommended as the indicator of choice for drinking-water (WHO, 1993 and Mays, 1996).

2.3.2 **Physico-Chemical Characteristics of Water**

Good quality water should not be offensive to the sense of touch, sight, smell and taste.

Physical properties of water comprises appearance, odour, taste, turbidity, temperature etc. The Chemical characteristics of natural water are a reflection of the soils and rocks with which the water has been in contact. In addition, agricultural and urban runoff, and municipal and industrial wastewater after treatment, impact the water quality. Microbial and chemical transformations also affect the chemical characteristics of water (Mays, 1996). Some of the parameters under study are described briefly below.

Temperature

Temperature is basically important for its effects on the chemistry, and biological reactions in the organisms in water. The temperature of freshwater normally varies from 0 to35°C (32 to 95°F), depending on the source, depth, and season. Cool water is generally more palatable than warm water. High water temperature enhances the growth of micro-organisms and leads to the speeding up of the chemical reactions in water, reduces the solubility of gases and amplifies the tastes and odours (Trivedy and Goel, 1986 and WHO, 1993).

Turbidity

The clarity of a natural body of water is an important determinant of its conditions and productivity. Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity is a measure of the optical property that causes, light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 1995).

pH

pH is the negative $\log_{10}$ of the hydrogen ion concentration in a solution. Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection and corrosion control, is pH-dependent. pH is used in alkalinity and carbondioxide measurements and many other acid-base equilibria. At a given temperature the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity (APHA, 1995).

Acidity

Acidity of a water is its quantitative capacity to react with a strong base to a designated pH. Acidity is a measure of an aggregate property of water, and strong mineral acids, weak acids such as carbonic and acetic, and hydrolyzing salts such as iron or aluminium sulfates may contribute to the acidity. Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation, and biological processes (APHA, 1995).

Alkalinity

Alkalinity of a water is its acid-neutralizing capacity. It is the sum of all the titratable bases. Alkalinity is a measure of an aggregate property of water. Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Carbonate, bicarbonate, hydroxide content and others contribute to the alkalinity (APHA, 1995).

Conductivity

Conductivity is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement. Solutions of most inorganic compounds are relatively good conductors, while molecules of organic compounds that do not dissociate in aqueous solution conduct a current very poorly, if at all (APHA, 1995).

Hardness

Hardness reflects the composite measure of the polyvalent cation concentration in water. Calcium and magnesium ions are the primary constituents of hardness. Originally, water hardness was understood to be a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by the calcium and magnesium ions present. Total hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per litre (APHA, 1995 and Mays 1996).

Calcium

The presence of calcium in water supplies results from passage through or over deposits of limestone, dolomite, gypsum, and gypsiferous shale. Small concentrations of calcium carbonate combat corrosion of metal pipes by laying down a protective coating. Calcium contributes to the total hardness of water. Chemical softening treatment, reverse osmosis, electrodialysis,

or ion exchange is used to reduce calcium and the associated hardness (APHA, 1995).

Magnesium

Magnesium is a common constituent of natural water. Magnesium salts are important contributors to the hardness of a water, and they break down when heated, forming scale in boilers. Concentrations greater than 125 mg/l also can have a cathartic and diuretic effect. Chemical softening, reverse osmosis, electrodialysis, or ion exchange reduces the magnesium and associated hardness to acceptable levels. (APHA, 1995).

Iron

Anaerobic groundwater may contain ferrous iron at concentrations of up to several milligrams per litre without discoloration or turbidity in the water when directly pumped from a well. On exposure to the atmosphere, however, the ferrous iron oxidizes to ferric iron, giving an objectionable reddish-brown colour to the water.

Iron also promotes the growth of "iron bacteria, and at levels above 0.3 mg/litre, iron stains laundry and plumbing fixtures. There is usually no noticeable taste at iron concentrations below 0.3 mg/litre, although turbidity and colour may develop (WHO, 1993).

Ammonia

Ammonia is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. Sewage has large quantities of nitrogenous matter, thus its disposal tends to increase the ammonia content of the waters. Thus, occurrence of ammonia in the waters can be accepted as the chemical evidence of organic pollution. Ammonia in higher concentration is toxic to humans and aquatic life. The toxicity of ammonia increase with pH because at higher pH most of the ammonia remains in the gaseous form. The decrease in pH decreases its toxicity due to conversion of ammonia into ammonium ion which is much less toxic than the gaseous form (Trivedy and Goel, 1986).

Chloride

Chloride, in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water

and wastewater. In potable water, the salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg Cl^-/l may have a detectable salty taste if the cation is sodium (APHA, 1995).

Dissolved Oxygen

Dissolved oxygen (DO) is important in natural water, because DO is required by many micro-organisms and fish in aquatic systems. DO also establishes an oxic environment, in which oxidized forms of many constituents in water are predominant. Respiration deficit or deplete the concentration of DO in water, and can result in anoxic or anaerobic conditions. Under anoxic conditions, reduced forms of chemical species are formed and frequently lead to the release of undesirable odours until oxic conditions develop. DO levels depend on the physical, chemical, and biochemical activities in the water body (Mays, 1996).

Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) represents the amount of oxygen required for the microbial decomposition of the organic matter in the water. Higher the BOD value, lower in the water quality. The BOD procedure, which is used extensively in monitoring water quality and bio-degradation of waste materials, is designed to determine how much oxygen is consumed by micro-organisms during oxidation of the organic matter present in the sample. The 5-day BOD (BOD_5) is most widely used. The BOD_5 of natural waters is related to the DO concentration, which is measured at zero time and after 5 days of incubation at 20°C. The difference is the DO used by the micro-organisms in the biochemical oxidation of organic matter (Atlas, 1989 and Mays, 1996).

2.4 Enterobacteriaceae

Enterobacteria are members of the family Enterobacteriaceae. These are Gram-negative, aerobic or facultatively anaerobic, non-spore-forming, often motile by peritrichous flagella or non-motile, catalase positive, oxidase negative bacilli. They reduce nitrates to nitrites, ferment glucose in peptone water with the production of either acid or acid and gas, and they breakdown glucose and other carbohydrates both fermentatively under anaerobic conditions and oxidatively under aerobic conditions, e.g. in the Hugh-Leifson test. They can be found in the intestinal tract of humans and animals, and in the soil and water. Some species are commensals and some are pathogenic for humans. The principal genera of Enterobacteriaceae of clinical interest include

Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Hafnia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella and *Yersinia* (Collee *et al.*, 1989; Greenwood *et al.*, 1992; Cheesbrough, 1993 and Chakraborty, 1995).

2.4.1 Waterborne Diseases

Infectious diseases caused by bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking water. The diseases are transmitted particularly through human and animal excreta, particularly faeces (WHO, 1993). Sewage is also a potential source of pathogenic micro-organisms. Once these organisms reach the water they can survive for several days and their survival is highly dependent on temperature, presence of organic material, and on the presence of other micro-organisms.

Table 1 : Survival time of some excreted pathogens in sewage contaminated water. *

Pathogens	Survival Times
Giardia lamblia	20 days
Entamoeba cysts	15 days
Ascaris egg	1 day
Escherichia coli	20 days
Salmonella typhi	30 days
Salmonella paratyphi	30 days
Vibrio cholerae	20 days
Hepatitis virus	60 days

* World Bank Technical paper No. 51, quoted by Sharma, 1993.

2.4.2 Outbreaks of Waterborne Diseases in Nepal

The WHO has estimated that up to 80% of all sickness and disease in he world is caused by inadequate sanitation, polluted water, or unavailability of water (Cheesbrough, 1993). Many outbreaks of waterborne diseases probably are not recognized; therefore, their incidence are not reported. But there are real incidents of waterborne disease, in which improvements in drinking water quality could have saved many lives.

Outbreak of waterborne epidemic is rampant in Nepal as in most of the third world

countries. Mortality and morbidity due to such diseases still top the list. Every year the onset of the epidemics come along with the monsoon. As it is mentioned in the UNICEF situation analysis (UNICEF, 1987), in Nepal water and hygienic related diseases, are responsible for 15% of all cases and 8% of all deaths in the general population. In 1985, over 50% of hospital patients in Nepal were found to be suffering from gastro-intestinal disorder normally caused by waterborne pathogens (ADB, 1985).

In 1990, cholera outbreak during summer hit different parts of the country including the capital city and caused an enormous loss of lives (DISVI, 1990). In Pokhara, more than 50% of the leading diseases causing morbidity were recorded to be waterborne. In Emergency Department of Pokhara Hospital 322 persons were registered only due to cholera in between June-July, 1990. In 1990, Public Health Division recorded 23,888 gastro-enteritis cases in 39 districts, maximum in the Kathmandu valley (8,437). In 1991 as well, the disease started to spring up at the beginning of the summer, striking badly the western, mid-western and central region of the country, while the eastern region was less affected. In between April to August, Public Health Division reported 43,520 gastro-enteritis cases with 1,252 deaths (ENPHO/DISVI, 1991). There was outbreak of gastro-enteritis in Bhaktapur district during summer in 1995 (ENPHO/DISVI, 1995). The recent outbreak of gastro-enteritis in eleven districts of the Kingdom has recorded 2,300 cases with 69 deaths in between March-April, 1998 (Gorkhapatra Daily, 1998).

2.5 Drinking Water Quality in Nepal

There have been a number of studies to assess the water quality intended for drinking in Nepal. Almost all the studies have found the water unsatisactory in terms of bacteriological quality. The trend of water pollution is increasing with time in all types of sources. Urban development in Nepal has been chaotic and unplanned (Sharma, 1987) and, along with increasing industrial and commercial activities, has led to a deterioration in the urban living environment (Joshi, 1987).

2.5.1 Studies on Microbial Quality

Today, most of 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 varying levels of treatment, bacteriological contamination remains high. The quality of water supplied from the centralized system is not always satisfactory. Frequent cases of contamination have been recorded (Joshi, 1993).

Sharma (1978) studied the quality of drinking water supplied to the households of the Kathmandu valley. Coliform tests were performed on water samples from 39 localities and result, showed that all the water samples had some degree of faecal contamination. The coliform densities ranged from 4-460col/100 ml. of water. In a follow-up study, Sharma (1986) found that the level of coliform contamination of drinking water in Kathmandu had significantly increased. He found the maximum bacterial level reached 4,800 col/100 ml of sample. Adhikari *et al.* (1986) carried out coliform tests on 100 drinking water samples collected from different areas on the Kathmandu valley and from different sources. Most of the unsatisfactory water samples had more than 1,800 col/100 ml of water. Joshi (1987) performed bacteriological tests of drinking water sources of two villages Central Nepal nearer to the capital city : Chaubas (Shivapuri) and Syabry (Langtang). He found the coliform count in the range of 5-100 col/ 100ml of water. In Chaubas, water from unprotected springs showed contamination within the range of 20-100 col/100ml. CEDA (1989) carried out bacteriological tests of water samples from different localities in Kathmandu. CEDA study found that all samples were contaminated with faecal materials. None of the tested tap and groundwater sources were safe for drinking.

ENPHO/DISVI (1990) conducted a study on water quality of 21 stone spouts of the Kathmandu city. Bacteriologically, samples from all the spouts have shown faecal contamination. The faecal coliform densities were observed in the range of 1 to 37,602 col/100 ml of water. 81% of the spouts showed very high contamination (> 100 col/100ml), and rest 19% exhibited less than 100 col/100ml. ENPHO/DISVI (1990) assessed water quality in Terai Tubewell Project in seven different localities of Morang, Biratnagar, Sunsari, Jhapa, Siraha and Saptari districts. A total of 164 water samples were tested, and 89.8% of samples from project tubewells and 85% from non-project tubewells showed less than 10 faecal coliforms per100ml water. Similarly, 93.3% of samples from project dugwells and all of non-project dugwells have more than 100 faecal coliforms per 100ml of water. ENPHO/DISVI (1991) analysed 11 water samples collected from different sources and different areas in Pokhara. The bacteriological result indicated that all the types of water to be contaminated, except two household samples. The density of faecal coliform bacteria ranged from 39-123 col/100ml of water. ENPHO/DISVI (1991) examined 107 water samples from tubewells dugwells, ponds and households in thirteen panchayats in Siraha district. Water from 34% of the sources were found free of faecal contamination, while rest 64% were all polluted. ENPHO/DISVI (1992) conducted a one year monitoring on microbiological quality of water supply in the Kathmandu city. 39 samples from 5 treatment plants and 172 samples from 37 public taps were examined from different localities. 7

samples that is, 18% from treatment plants were found contaminated with an average faecal coliform of 4 col/100ml. Similarly, 50% samples from public taps were found contaminated. The bacterial densities in contaminated samples ranged from 1 to TNTC col/100ml.

Pradhananga *et al.* (1993) examined water samples from 6 different stone spouts around Pashupati area on three occasions during 1992. Average values of coliform at Ban Binayak (44 col/100ml), Arun Dhara (88 col/100ml), Barun Dhara (43col/100ml), Ganga Hiti (10 col/100ml), Mitra Dhara (8 col/100ml), and Ba Hiti (61 col/100ml) were recorded.

In Patan, out of 49 samples supplied by centralized system from 9 different sites, 24 samples (49%) were found to be bacterial contaminated. Sanepa, Lagankhel, Mangal Bazar and Gwarko were recorded as most polluted sites. During the month of May in 1995, percentage of contamination level in Kathmandu city water supply reached maximum (about 88%) and bacterial densities also increased up to more than 3,000 col/100 ml (ENPHO/DISVI,1995). Joshi *et al.* (1992) reported the water quality of stone spouts at Patan area. Water from 21 spouts not tested, 3 polluted, 5 slightly contaminated, 2 excellent, 1 good, and not mentioned about 2 spouts. The faecal coliform densities ranged from 0-4,400 col/100ml of sample. Water samples tested from 29 wells in four wards (Ward No. 6,7,8 and 22) in Urban Patan in 1991 by CDHP were found unsatisfactory having high coliform count (Lewis, 1995).

Sharma (1993) carried out bacteriological examination of the potable water of different urban and rural areas of Nepal. He recorded the maximum densities of colifroms and faecal coliforms as 4,800 col/100ml and 240 col/100 ml respectively in Kathmandu, 240 col/100ml and 93/100ml in Hetauda, 4,800 col/100ml and 4,800 col/100ml in Birgunj, Pokhara and Biratnagar. He also isolated some enterobacteria from water samples. The study indicated that in rural area 33.3 to 16.7% of water samples and in urban area 70 to 100% samples were found to be contaminated with coliform bacteria.

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

Thapa (1997) examined water quality of nine different sites in Baluwa VDC, near to the Kathmandu city. Water samples were collected during three seasons and from different sources. All the samples were contaminated during all the seasons. The bacterial densities ranged from 43-210 col/100ml during winter, 75-240 col/100ml during summer and 150-460 col/100 ml during rainy season.

2.5.2 Studies on Physico-Chemical Parameters

Sharma (1986) studied physico-chemical parameters of tap water samples from 51 different areas in Kathmandu. He noted little variation in the chemical content of drinking water supplied to different localities in Kathmandu. The pH content ranged from 6.5 to 7.5, while $CaC0_3$ content varied from 26 to 30 mg/l. Total hardness was almost similar from one location to another. The chemical constituents tested were found to be within the standards prescribed by WHO, 1984.

DISVI (1990) studied water quality in seven rural areas of Ilam in eastern Nepal and found pH (acidic), total hardness and chloride values outside the acceptable limit, ammonia (NH_3-N), and iron (Fe) and within the standards set by WHO, 1984. ENPHO/DISVI (1990) recorded the values for turbidity and total hardness from all the 21 stone spouts in Kathmandu lie within the WHO standard. Water from most of the taps were almost neutral as indicated by the average pH values, and 2 samples were slightly acidic. The values for conductivity and chloride of all the taps were within the maximum permissible limit. In the study, 71% of the taps were recorded to exceed the safety limit for ammonia. The concentrations of iron were noted fairly low except for one tap (1.28 mg/l). ENPHO/DISVI (1990) examined water quality in Terai Tubewell Project in seven rural areas of the Eastern Development Region of Nepal. Most of the parameters (appearance, odour, pH, conductivity, calcium, magnesium, chloride, ammonia, and oxygen consumed) were found within the WHO guidelines, while iron and hardness exceeded the value. ENPHO/DISVI (1991) analyzed water quality in Siraha district. Average values for pH, total hardness and chloride were found within WHO acceptable limit. Average conductivity ranged from 461-1,313 uS/cm, ammonia from 0.2-1.1 mg/l and iron from 0.5-7.1 mg/l.

Pradhananga *et al.* (1993) examined water samples of stone taps of Pashupati area in 1990 and 1992. In 1990 the average pH, N- ammonia and iron values ranged from 6.3-6.5,

<0.05-0.56mg/l and 0.05-0.84mg/l, respectively. The average values for conductivity, hardness and chloride lie within the WHO permissible level. Likewise, in 1992, the temperature and pH ranged from 21.4-23.4°C and 6.3-6.7 respectively. Parameters like conductivity, hardness, chloride and turbidity were recorded within permissible level. Average values for iron and N-ammonia ranged from 0.2-1.9 mg/l and < 0.01-3.02 mg/l respectively.

Ghimire (1996) assessed 11 groundwater samples from Patan area in two seasons. In rainy season, the pH and temperature ranged from 5.6-6.3 and 19.7-22.5°C respectively. Conductivity, hardness and N- ammonia, for all samples were found to lie within WHO permissible level. The values for chloride and ammonia ranged from 60-288 mg/l, and > 1.5 mg/l respectively. Similarly, in summer reason, pH and temperature ranged from 5.9-6.7 and 21-22.7°C. The values for conductivity and hardness were found within the WHO limit, and chloride, N-ammonia, total iron, calcium and magnesium ranged from 50-324 mg/l, 0.13->1.5mg/l, <0.05-5.15mg/l, 33.6-83.2 mg/l and 4.38-35.57 mg/l respectively.

Thapa (1997) recorded most of the parameters analysed within WHO standard for drinking water except BOD value of some samples. Temperature, pH, total hardness, chloride were found within safe limits set by WHO, 1993.

2.6 Works on Drinking Water Quality in Other Countries

Antai (1987) examined the bacteriological quality of some rural water supplies in Port Harcourt, Nigeria and suggested that the supplies were unsatisfactory as judged by the presence of total coliforms, and faecal coliforms. The most frequently isolated coliforms were *E. coli*, *E. aerogenes*, and *K. pneumoniae*. Besides these, *S. aureus* and *P. aeruginosa* were also recovered.

Yulug and Tug (1988) analysed 150 well water samples from different parts of Ankara (Turkey). Only 20 samples (13.3%) were drinkable. In 117 samples (78%), 120 coliform bacteria strains were isolated, of these 80 were *E. coli*, 27 *Enterobacter* and 13 *Citrobacter* .

Ibiebele and Sokari (1989) assessed 108 raw water samples from 36 wells at 9 shanty settlements around Port Harcourt, Nigeria for bacteriological quality. The organisms isolated include *E. coli*, *Klebsiella sp.* , *Proteus sp.* , *Serratia sp.*, *Pseudomonas sp.*, and others.

Hosny *et al.* (1990) examined a total of 111 water samples from 15 wells located at three water treatment plants in Cairo, Egypt. Coliforms were detected in 29 (80.6%), 30 (100%) and 40 (88.9%) of water samples taken from three different plants. Their densities were in the range 1-35 col/100 ml. Faecal coliforms were detected in 39.6% of samples.

Combarro *et al.* (1988) analysed 80 water samples from rural wells of Galicia, Spain. Total coliforms (63.75%), faecal coliforms (23.75%), and *E. coli* (21.25%) were found. The study showed 25% of the samples were drinkable, 21.4% could be acceptable after chlorination and 53.6% were inadequate presumably due to organic matter contamination.

Wadud *et al.* (1992) carried out a study on drinking water of 12 villages near Peshawar (Pakistan) from different sources. *E. coli, Salmonella,* and *Shigella* were detected from 120 samples. The most prevalent was the coliform bacteria. Almost all the samples showed bacterial contamination beyond the standard values.

Khan and Khan (1992) conducted examination of 40 water samples from various sources from the villages and towns of Mardan Division, Pakistan. The pH (6.23-9.28), alkalinity (112.0-1080.0 mg $CaC0_3$/l), conductivity (98.0-1200.0 μS/cm) and chlorides (54.5-599.0mg/l) were studied.

Mahasneh (1992) investigated the occurrence of faecal indicator bacteria in urban and rural drinking water sources at thirty sites of spring waters in Jordan. The most probable number (MPN) of the total and faecal coliform counts ranged from 200-1,600/100ml.

Pathak *et al.* (1993) carried out the bacteriological analysis of 50 drinking water samples from various sources in different parts of Lucknow city. 56% and 44% of samples showed coliforms (> 10/100ml) and faecal coliforms (>1/100ml), respectively. *E. coli* were isolated from 13 samples, and all the isolates were found to be enterotoxigenic.

Eldin *et al.* (1993) tested the quality of water from 388 wells in 6 regions in the Kingdom of Saudi Arabia. 21.4% of samples showed the presence of faecal coliforms. About 16% of the wells had NH_4^+ levels above the WHO limit.

Somasundaram *et al.* (1993) conducted a study on the water quality in the wells of the Madras, India. Of the 93 groundwater samples, 25% exceeded the calcium limit, 11% the magnesium limit, relative to Indian domestic water standards. Microbes were found in several of the wells.

Pathak and Gopal (1994) examined a total of 89 drinking water samples from different natural sources in India for bacterial contamination. About 49.4% of samples showed presence of coliforms . Recovery of *E. coli* was maximum followed by *Citrobacter, Klebsiella*, and *Enterobacter.*

Rai and Sharma (1995) carried out a study on groundwater in rural areas of North-West Uttar Pradesh, India. Total coliforms, faecal coliforms and *E. coli type 1* were estimated as 4460, 1480 and 305 col/100ml of water respectively in 15 well water samples collected from rural areas of Bareilly and Nainital districts. Few samples were free from *E. coli type 1.*

2.7 Antibiotics and Bacterial Resistance to Antibiotics

An antibiotic refers to a substance produced by a micro-organism or to a similar substance (produced wholly or partly by chemical synthesis) which, in low concentrations, inhibits the growth of other micro-organisms (Hugo and Russell, 1981).

In 1929 Alexander Fleming discovered the first antibiotic, penicillin, a metabolic product of *Penicillium sp.* (*Penicillium notatum*). This discovery opened the era of antibiotics (Pelczar *et al.* , 1986).

Antimicrobial substances have proven an effective weapon against bacterial contamination and infection. But, the resistance, activity, stability and selective toxicity of some of these substances against certain micro-organisms have noted to be lost. Most of the microbial resistance which is now making it difficult to treat some infectious diseases is of genetic origin and transferable between species and genera of bacteria. Acquired antimicrobial resistance is a worldwide problem.(Cheesbrough, 1993). Bacteria can develop a large variety of mechanisms for antibiotic resistance and they also possess genetic mechanism for the spread of this resistance. Antibiotic resistant plasmids (R-Plasmids) are found in several bacterial genera both gram-positive and gram-negative. Transmissible plasmid-borne antibiotics resistance was first dis-

covered in multi-resistant *Shigella* strains in Japan in 1959. Since then, this phenomenon has been reported all over the world and is now ubiquitous among the both pathogenic and commensal bacteria. Repeated exposure of bacteria to antibiotics may lead to the development of resistant bacterial strains. Some bacterial cells under go genetic mutations or produce enzymes that destroy or inactivate antimicrobials, as a consequence, make them resistant to the antibiotics. In this way, they live and multiply in the presence of the drug giving rise to a resistant strain (Crumplin and Odell, 1987).

2.7.1 Studies on Bacterial Resistance to Antibiotics

Chugh and Suheir (1983) assessed drug resistance among *Salmonella sp.* prevalent in Kuwait during 1979-1980. Out of 345 isolates, only 9.6% were sensitive to all the 14 drugs tested. There was resistance to tetracycline (69%), kanamycin (61%), ampicillin (56%) and chloramphenicol (38%). Multiple drug resistance was observed in 71% of isolates and many of them were resistant to five or more drugs.

Antai (1987) found 17.5-27.2% of *E. coli* strains isolated from rural water supplies in Port Harcourt, Nigeria resistant to three or more antibiotics. *E. coli* recovered from wells exhibited the greatest degree of multiple resistance. Some strains were resistant to all the six antibiotics tested.

El-Zanfaly *et al.* (1987) conducted a study on the incidence of antibiotic resistant bacteria in drinking water in Cairo, Egypt. Most isolated strains appeared to be ampicillin resistant (89.7%). Out of total isolates 62.4-98% exhibited resistance to two or more antibiotics, and a total of 363 multiple-antibiotic resistant strains were identified.

Hosny *et al.* (1988) examined 101 isolates from underground water in Cairo (Egypt) for their resistance towards four antibiotics. He found 32 and 18 isolates resistant to tetracycline and chloramphenicol , respectively.

Ramtete *et al.* (1991) studied coliform isolates from different drinking water sources for antibiotic resistance. They found that resistance to ampicillin was found more prevalent among the coliform isolates.

Pandey and Musarrat (1993) studied antibiotic resistance of coliforms bacteria isolated from drinking water in the urban area of Aligarh city, India. Multiple-drug resistant *E. coli* was isolated, and the frequency of drug resistance was determined to be significantly high to ampicillin and tetracycline.

Pathak and Gopal (1994) studied antibiotic resistance among coliforms isolated from about 49.4% of 89 drinking water samples in India. Out of total coliform isolates 36.4% exhibited resistance for varying number of antibiotics (12nos.). Resistance for ampicillin (56.8%) was found maximum. Half of isolates exhibited multiple-antibiotic resistance.

Bissonnette *et al.* (1995) examined 265 isolates from rural groundwater supplies in West Virginia (USA) for resistance to 16 antibiotics. All of the non-coliforms and 87% of the coliforms were resistant to at least one antibiotic. Percentage resistant to ampicillin, nitrofurantoin, tetracycline, chloramphenicol and nalidixic acid were observed as 69.4, 47.7, 32.3, 16.9 and 12.0 respectively, while below 10.0 for amikacin and gentamycin.

2.8 Oligodynamic Action

Extremely small amounts of certain metals, particularly silver, can exert a lethal effect upon bacteria; this is designated oligodynamic action from the two Greek words *oligos*, meaning "*small*," and *dynamis*, meaning "*power*". A zone of inhibition (no growth) surrounding the metal on an inoculated plate demonstrate the phenomenon. The amount of metal (silver or copper) needed for this inhibitory effect is extremely small, only a few parts per million. Numerous compounds of heavy metals have germicidal or antiseptic activity. The most prominent antimicrobial compounds of heavy metals are those of mercury, silver and copper. One mode of action of heavy metals and their compounds is through protein denaturation (Pelczar *et al.*, 1981).

The high affinity of cellular proteins for the metallic ions results in the death of cells due to the cumulative effects of ions within the cell. This characterisic of heavy metals has been applied to water purification, ointment manufacture and the treatment of bandages and fabrics (Benson, 1979).

2.8.1 Studies on Oligodynamic Action

The ability of silver and copper vessels to purify water has been reported in several reports. Concerning bactericidal action of silver, Clark (1956) has reported that filter candles coated with silver has germicidal action.

Out of two water samples (stored in copper pots) tested in Pokhara, one was noted to be free of the bacteria and one less contaminated. The water from aluminium pot showed high bacterial contamination. Similarly, in previous study for UNICEF in Ilam (1990), water stored in copper pots were comparatively less contaminated indicating the potential role of the pot material in decreasing bacterial density (ENPHO/DISVI, 1991).

Shahi *et al.* (1996) tested five types of traditional Nepalese pots for their bactericidal action against *E. coli* (ATCC 25922). Copper, silver and brass pots were found to be very effective, steel and aluminium pots less effective where as earthern pot ineffective for their bactericidal action.

2.9 Water Quantity and Quality, Sanitation, Health and Education

A convenient supply of safe water and the sanitary disposal of human wastes are essential ingredients of a healthy, productive life. But, rapid population growth, and uncontrolled urbanization and industrialisation have increased the pressure upon limited water supplies and upon systems of waste disposal. It is sad that the vast majority of the world's populations have neither access to safe water nor good sanitation facilities (Sidwick, 1984).

It is estimated that three quarters of the people in the developing world do not have reasonably safe supply of drinking water. In a 1975 estimate, only 22% (313 million) out of 1,422.72 million rural people in 75 developing countries (excluding China) had reasonable access to safe water. The remaining 78% have to rely on various sources of water supply, mostly contaminated and unsafe (Obeng, 1982). Sidwick (1984) has written that over 1800M people in developing countries lack a reasonable supply of water and at least 2400M people have no sanitation facilities. In Nepal only 3% of the urban population have access to a sewerage system with another 10% having access to some sort of excreta-disposal system, often a totally inadequate one; the remaining 87% of the urban dwellers have nothing; and in urban core-areas there are no fields. The United Nations declared the 1980s the International Drinking Water

Supply and Sanitation Decade (IDWSSD) with the goal of safe water and sanitary facilities for all by 1990. Its aims and objectives remain as commendable and essential as they were in 1980 for a variety of reasons. Some constraints for developing countries include an insufficient number of trained professionals. insufficient funding, inadequate operation and maintenance, ineffective logistics, insufficient health education effects, lack of planning and design etc. (Mays, 1996).

WHO gave the following water and sanitation data (WHO 1987, quoted by Cairncross 1989) :

Table 2 : Percentage of population in developing countries with adequate access to facilities.

	1970	1980	1985
Urban water supply	65%	74%	77%
Rural water supply	13%	33%	41%
Urban sanitation	54%	50%	62%
Rural sanitation	9%	13%	18%

Ingestion of contaminated drinking water is not the only means of transmission of disease, but this route includes some of the most lethal diseases : typhoid, cholera, dysentery, hepatitis, and the various diarrhoeal illnesses. Millions die each year in the developing countries from diseases spread by contaminated water. 80% of all sickness in the world is attributable to inadequate water or sanitation, according to WHO estimates. Over 1.5 billion people in the Third World countries of Asia, Africa, and Latin America-75% of the population-lack a supply of safe water for drinking and washing and/or adequate sanitation (Morrison, 1984)

Interventions to improve water quality and quantity, excreta disposal, along with hygiene education associated with better hygiene practices, produce greater impacts on general public health. If there is water in sufficient quantity the agent of infection will be diluted to safe levels (Elemendorf and Isely, 1982). The combined effects of adequate water supply and improved water quality have been shown to produce an average of 25% reduction in water-related diseases in recent years (WHO, 1993). Examples of hygiene behaviour cited by Cairncross (1990) include the washing of hands, food and utensils, and the disposal of children's excreta. Khan (1982) reported a 69% reduction in secondary infection of *Shigella* as a result of increased hand-washing with soap and water, and that washing without soap produced far less impact (quoted by Kolsky, 1993). Aung Myo Han and Thein Hlaing (1989) reported a 30% reduction in diarrhoeal disease among children under 5 years old as a result of

a similar intervention to increase hand- washing.

Throughout the developing world women and children still have to "fetch" water daily, sometimes from very long distances. It is women who form a constant link in the chain of contamination from faeces to fingers to food and who can break the chain by latrine use, hand-washing, and protection of left-over food (Elemendorf and Isely, 1982). The water source may or may not be safe, but good quality water at source may become contaminated in transit, or in domestic water storage before use (Narayan-Parker 1988; Prakash 1989). Unless women and children become convinced of the need to change the way they handle drinking-water in the home it will continue to be polluted, despite improvements at source (Narayan-Parker, 1988). In many instances children who have been tought proper sanitary measures in school become, in effect, the teachers of their parents and other family members, and can help considerably in changing customs and behaviours (Elemendorf and Isely, 1982).

The water quality status in Nepal can be best understood by the situation analysis report of UNICEF (1987) which states that "water and hygienic related diseases are responsible for 15% of all cases and 8% of all deaths in the general population. From birth to the age of four years, however, water related diseases are responsible for 41% of all cases and 32% of all the number of deaths." Many studies have revealed to very high content of coliform bacteria in potable water in both Terai and hilly regions of Nepal. In rural area 16.7% to 33.3% and in urban area 70 to 100% water samples are found to be contaminated with coliform bacteria. Almost all piped water supply are contaminated during rainy/ summer season, and water quality is far from satisfactory in almost all localities of urban area. From sanitary point of view the sewerage disposal system of Kathmandu Valley and other cities of Nepal are very unhygienic as a result of which diarrhoea and dysentery associated with polluted water is very common in the cities. Serious efforts are needed to reduce bacterial contamination in potable water (Sharma, 1994).

CHAPTER - THREE

METHODOLOGY

3.1 Study Area and Sample Collection

This study was conducted in urban area of Patan, one of the historical cities of the Kathmandu valley. This ancient city of Patan, also known as the city of artists, is situated five kilometres south-east of Kathmandu across the southern bank of the river Bagmati. There are 35 stone spouts (but mentioned 34 by Joshi, 1993) and hundreds of dug wells in the city.

Water samples were randomly collected from different groundwater sources. A total of 70 samples from 5 shallow pumps, 3 shallow wells, 14 stone spouts and 48 dug wells were collected. In this study, the sources into which a pipe is inserted underground (not more than 15 metres deep) and water is drawn with the help of hand pump are considered as *Shallow Pumps*. Hand dug wells which are protected by sealing the well mouth with a cover or lid and water is collected either with the help of hand pump or using a bucket and rope by opening the lid are considered as *Protected Wells*. Open hand dug wells from which water is raised to the surface by using a bucket and rope are considered as *Unprotected Wells*. The wells which are very shallow (either one or two metres deep) are considered as *Shallow Wells*. The samples were collected during the period of October (fourth week) to November (third week) 1997.

3.2 Survey of Water Supplies and Questionnaires

This study was designed for collection of both quantitative and qualitative information about the sample sources during sampling period. A format or questionnaire was prepared to collect physical informations about the water supplies and the surrounding areas, family size, total number of people dependent on the water sources, reasons for using groundwater supplies, pot types used to collect and store drinking water, water purification or treatment methods used by community people at source and at home, sanitary/ sewage conditions and consequences of the pollution with respect to the health related problems and people's perception about existing water quality and health etc. In this study, Sanitary condition of confirmed aquifers supplying water to the stone spouts was also studied by direct field visit.

Some of the physical informations such as depth and age of the sources were collected

by asking with the elderly inhabitants who lived closest to each well or stone spout. The information about the sanitary condition of the water supplies, possible sources of pollution, type of sources, protected or unprotected, pot types used to collect water for drinking were collected by direct field observations. Likewise, men and women who had come to collect water in the sources were interviewed after telling them in detail about the objectives of the study to gather information about what the water was like in their opinion, what it was used for, how many used it, pot types used to store it at home, community level treatment at the source and methods of home treatment for drinking purpose, water-borne disease cases etc.

3.3 Sampling Sites

Following water sources were selected for this study.

S.N.	Code No.	Sources	Location and Ward No.
1.	SP1	Shallow Pump	Sanepa, Ring Road -2
2.	SP2	"	Sikabahil, Shankhamul -22
3.	SP3	"	Gusingal, Kopundole -1
4.	SP4	"	Kopundole -10
5.	SP5	"	Jwagal Tole -10
6.	SW1	Shallow Well	Satdobato -17
7.	SW2	"	Mahadevsthan,Shankhamul -9
8.	SW3	"	Shree Ram Mandir -2
9.	SS1	Stone Spout (Lun Hiti)	Sundhara -6
10.	SS2	" (Manga Hiti)	Mangal Bazar -11
11.	SS3	" (Makah Hiti)	Gui Tole -8
12.	SS4	" (Sinci Hiti)	Sincha -17
13.	SS5	" (Chyasa Hiti)	Chyasal -9
14.	SS6	" (Alkva Hiti)	Alko -22
15.	SS7	" (Konti Hiti)	Kumbeshwor -22
16.	SS8	" (Tanga Hiti)	Tangal -12
17.	SS9	" (Tapah Hiti)	Tapaha Hiti -22
18.	SS10	" (Pulchowk Hiti)	Pulchowk -3

S.N.	Code No.	Sources	Location and Ward No.
19.	SS11	" (Iku Hiti)	Dhobighat -4
20.	SS12	" (Bhole Hiti)	Bhole -8
21.	SS13	" (Jawalakhyo Hiti)	Jawalakhel -4
22.	SS14	" (Nah Hiti)	Naricha -6
23.	WC1	Protected Well	Patan Dhoka -22
24.	WC2	"	Ilachhen -17
25.	WC3	"	Kusunti -13
26.	WC4	"	Bulkumari -8
27.	WC5	"	Saugal (Well No.2) -10
28.	WC6	"	Machagal, Chakupat -22
29.	WC7	"	Lonhal Tole -9
30.	WC8	"	Bhindhyolachhi -11
31.	WC9	"	Jenbahal -19
32.	WC10	"	Taphalohn -5
33.	WC11	"	Harihar Bhawan, Inar -10
34.	WC12	"	Hakha Tole -11
35.	WC13	"	Saugal Chowk -12
36.	WC14	"	Sontha -18
37.	WC15	"	Bhanimandap -4
38.	WC16	"	Man Bhawan -5
39.	WC17	"	Tyagal Tole -17
40.	WC18	"	Munani -17
41.	WC19	"	Sanepa, Nayan Basti -2
42.	WC20	"	Chhenko Tole -22
43.	WO1	Unprotected Well	Nakabahil -16
44.	WO2	"	Chhayabahal -21
45.	WO3	"	Pilachhen Tole -7
46.	WO4	"	Nabahal -20
47.	WO5	"	Sako Tole -7

S.N.	Code No.	Sources	Location and Ward No.
47.	WO5	"	Sako Tole -7
48.	WO6	"	Bakhunbahal -11
49.	WO7	"	Ilanani -16
50.	WO8	"	Chanki Tole -7
51.	WO9	"	Subahal -8
52.	WO10	"	Tunchhi Galli -11
53.	WO11	"	Gujibahal -6
54.	WO12	"	Bhilachhen -9
55.	WO13	"	Wanibahal -18
56.	WO14	"	Dupat Tole -7
57.	WO15	"	Purnachandi Chowk -20
58.	WO16	"	Pulchowk Lachhi -3
59.	WO17	"	Nayaga Tole -20
60.	WO18	"	Kopundole -10
61.	WO19	"	Ikhalakhu, Nhu Tunchhi -18
62.	WO20	"	Sumangal Vihar -6
63.	WO21	"	Jhamsikhel, Ganesthan -3
64.	WO22	"	Sanepa -2
65.	WO23	"	Prayag Pokhari -6
66.	WO24	"	Gachhen -9
67.	WO25	"	Satdobato -15
68.	WO26	"	Mahalaxmi -15
69.	WO27	"	Pulchowk (CPS) -4
70.	WO28	"	Lagankhel, Podep -5

3.3.1 Collection, Transportation and Preservation of Samples

Sample collection

In this study, clean and presterilized (at 121°C for 15 mins. at 15 lb/in^2) and paper wrapped glass bottles were used for sampling. Water was collected in four bottles (one for bacteriologi-

Sample collection from stone spout

The bottle was held by the base in one hand while the other hand was used to remove and replace the screw cap or the stopper. Water was collected facing the mouth of the bottle towards the opposite direction of the water current, and then tilting the neck of the bottle slightly upwards to let it fill completely before carefully replacing the cap or stopper.

Sample collection from shallow pump or tubewell or covered well

The handpump was continuously operated for 5 minutes. The mouth of the pump was sterilized by igniting it after application of alcohol with a piece of cotton wool. Water was allowed to waste for sometime and then collected into the sampling bottles, and replaced the caps carefully.

Sample collection from an open well or shallow well

Sample bottle was tied on to a strong string and a piece of metal weighing about 500 grams as the weight was hung just below the bottle. The cap was removed and lowered the bottle into the well to a depth of about 1 metre. When no more air bubbles rose the surface, the bottle was raised out of the well and the cap was carefully replaced. In all cases the sample bottle was labelled with the sample code number immediately after filling it with the water sample.

Transportation and Preservation of Sample

Water samples should be transported and analyzed as quickly as possible to the laboratory and on arrival at the laboratory. In this study, water sample was subjected to microbial examination within one hour of collection. In some cases, when immediate analysis was not possible the samples were preserved at 4°C.

3.4 Microbilogical Examination of Water Sample

In this study, total coliforms and faecal coliforms were enumerated by the membrane filtration (MF) technique as described in APHA, 1995.

3.4.1 Membrane Filtration Technique

In this method, a measured volume of the water sample is filtered through a membrane filter with a pore size small enough to retain the indicator bacteria to be counted. The standard volume to be filtered for drinking water samples is 100 ml. This may be distributed among multiple membranes if necessary. The membrane is then placed and incubated on a selective, indicator medium, so that the indicator bacteria grow into colonies on its upper surface. The colonies which are recognized by their colour, morphology, and ability to grow on the selective medium, are counted. (Collee *et al.*, 1989 and APHA, 1995). The membrane filtration technique is recommended for its accuracy and speed of results, and also for its adaptability to incubation temperature and growth medium to improve growth and recovery of bacteria (Cheesbrough, 1993).

3.4.1.1 Standard Total Coliform Membrane Filter Procedure

(i) First of all, the sterile filter holder with stopper was assembled on the filter flask.

(ii) Using sterile blunt-edged forceps, a sterile membrane filter of pore size 0.45 μm (grid side up) was placed over the porous disk in such a way that it overlaps the entire circumference of the sintered filterable area.

(iii) The sterile funnel was securely placed on the filter base with the help of anodized aluminium spring clamp.

(iv) The sample of water was well mixed by inverting the bottle several times, and then the requisite volume of the water sample was poured or pipetted into the funnel.

(v) The sample was slowly filtered under partial vacuum by using electric vaccum pump. With filter still in place, the interior surface of the funnel was rinsed by 20-to 30 ml portions of sterile water.

(vi) The funnel was removed and the membrane was directly transferred, keeping its upper side upwards, on to a plate of M-Endo agar with the help of sterile flat-ended forceps. Care was taken not to entrap air bubbles between the membrane and the medium.

(vii) The petriplate was incubated for 24 hrs at 37°C in inverted position.

Enumeration of Coliforms

The typical coliform colony has a pink to dark-red colour with a metallic surface sheen. The sheen area may vary in size from a small pinhead to complete coverage of the colony surface. Atypical coliform colonies can be dark red or nucleated without sheen. Colonies that

lack sheen may be pink, red, white, or colourless and are considered to be noncoliforms (APHA, 1995). All sheen-producing colonies were counted after completion of incubation period.

Calculation of Coliform Density

The count was computed using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane, by the following equation (APHA, 1995).

$$\text{Total coliform colonies / 100 ml} = \frac{\text{coliform colonies counted}}{\text{ml sample filtered}} \times 100$$

3.4.1.2 **Faecal Coliform Membrane Filter Procedure**

The MF technique for the isolation of faecal coliforms from drinking water samples is similar to that for total coliforms except in the use of culture medium and incubation temperature. The faecal coliform MF procedure uses an enriched lactose medium and incubation temperature of 44.5 ± 0.2°C for selectivity (APHA, 1995). In this study, M-FC agar was used for the growth of faecal coliforms at 44.5°C for 24 hrs.

Enumeration of Faecal Coliforms

Colonies produced by faecal coliform bacteria on M-FC medium are various shades of blue. Nonfaecal coliform colonies are gray to cream-coloured (APHA, 1995). All the characteristic colonies for faecal coliforms were counted.

Caculation of Faecal Coliform Density

The density was computed using membrane filters with 20 to 60 faecal coliform colonies per membrane, by the following equation (APHA, 1995).

$$\text{Faecal coliform colonies/ 100 ml} = \frac{\text{Faecal coliform colonies counted}}{\text{ml sample filtered}} \times 100$$

3.5 **Isolation of Enterobacteria**

The enterobacteria present in the water sample were isolated by pour plate method using xylose lysine deoxycholate (XLD) agar, which permits the growth of almost all enteric bacteria.

Pour Plate Method

The pour plate method is simple to perform which involves inoculating water sample or its dilution on suitable medium in the petriplate and counting the colonies developed after the designated incubation time. In preparing plates, an appropriate dilution of sample is made that will yield 30 to 300 colonies per plate (APHA,1995).

Procedure

1. The water sample was thoroughly mixed by inverting the bottle several times, and then an appropriate serial dilution was made.
2. One ml from selected dilution was aseptically pipetted into a sterile petriplate.
3. Sterile liquefied xylose lysine deoxycholate (XLD) media maintained at 45°C in a water bath was poured into the plate, previously placed on a level surface, to form a layer 3-4 mm thick by gently lifting cover just high enough to pour.
4. Melted medium was thoroughly mixed with test portion in petriplate, taking care not to splash mixture over the edge, by rotating the plate first in one direction and then in the opposite direction.
5. The plates were allowed to solidify and then incubated at 37°C at inverted position for 24 hrs. The varies colonies grown after incubation were transferred onto nutrient agar (NA) slant for preservation.

3.5.1 **Detection of *Salmonella* species**

This group of bacteria is supposed to be very low in water samples. Thus, Selenite F broth was used as an enrichment medium for the isolation of *Salmonella sp.* in this study. The selenite in this enrichment medium inhibits coliform bacilli while permitting *Salmonella* and many *Shigella* to grow (Cheesbrough, 1993).

Enrichment Procedure

Several loopfuls of water sample was inoculated into Selenite F broth, and mixed thoroughly. It was then incubated at 37°C for overnight, and a loopful of the upper part of the broth was subcultured on a selective enteric medium, Salmonella - Shigella (SS) agar. The plate was incubated at 37°C for 24 hrs.

3.6 Isolation and Identification of Bacteria

Sheen - producing colonies and all colonies with different characteristics from M-Endo agar and XLD agar were streaked onto NA for purification. Isolated colonies were streaked onto NA slants and preserved at 4°C. Bacteria isolated on respective selective or differential media were identified on the basis of their colonial characteristics, morphological characteristics and biochemical properties. Identification was carried out following Bergey's Manual of Systematic Bacteriology (1984).

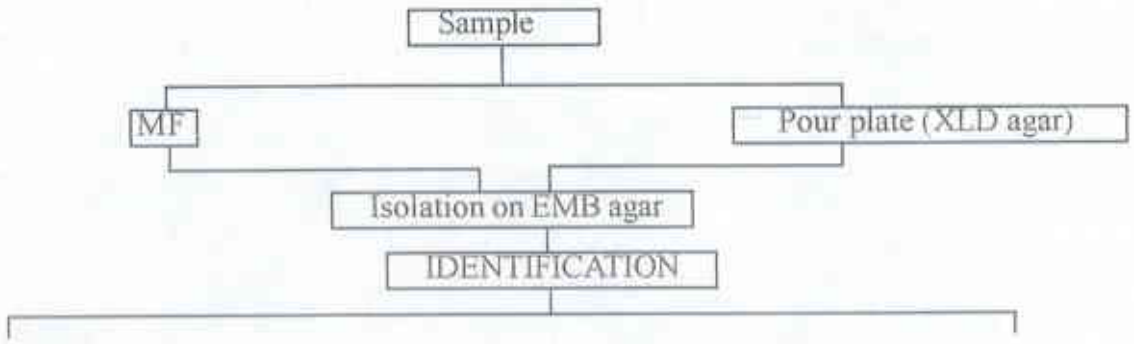
Study of Biochemical Tests

Biochemical tests are based on the ability of micro-organisms to produce enzymes thus utilizing different substrates of their environment. The test describes the ability of organisms to survive in the environment. The isolated pure colonies were inoculated into different biochemical media for different tests as described by Cheesbrough, 1993.

Table 3 : Biochemical Tests Performed for Identification of Enterobacteria Isolates

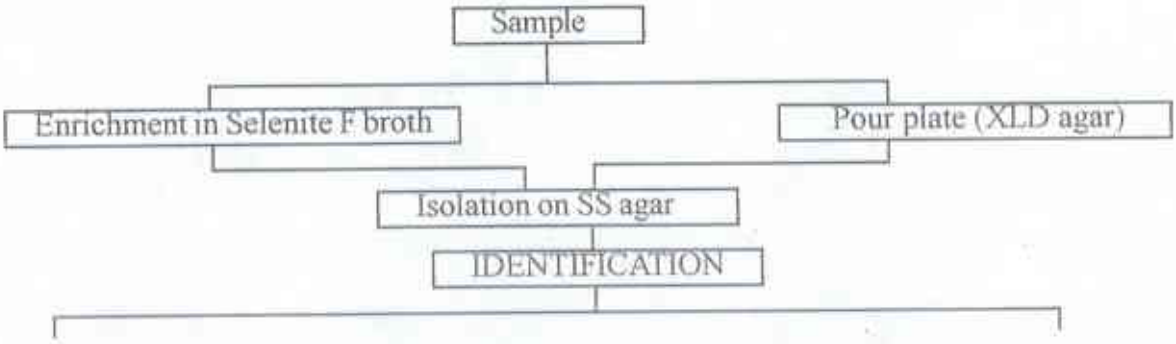
S.N.	Biochemical media	Tests
1.	3% H_2O_2	Catalase
2.	1% Tetramethyl-p-phenylene-diamine dihydrochloride	Oxidase
3.	Sulfide-Indole-Motility (SIM) Medium	H_2S and indole production, and motility
4.	Glucose phosphate peptone water	(a) Production of acid during fermentation of glucose (MR) (b)Acetoin production during carbohydrates fermentation (V-P)
5.	Simmon's Citrate Agar	Citrate utilization
6.	Triple Sugar Iron (TSI) agar	Fermentation of dextrose, lactose and sucrose, H_2S and gas production
7.	Christensen's Urea Broth	Urease production
8.	Nitrate Broth	Nitrate reduction
9.	Hugh and Leifson medium	Aerobic or anaerobic utilization of carbohydrates

FLOW CHART FOR IDENTIFICATION OF ***E. coli***



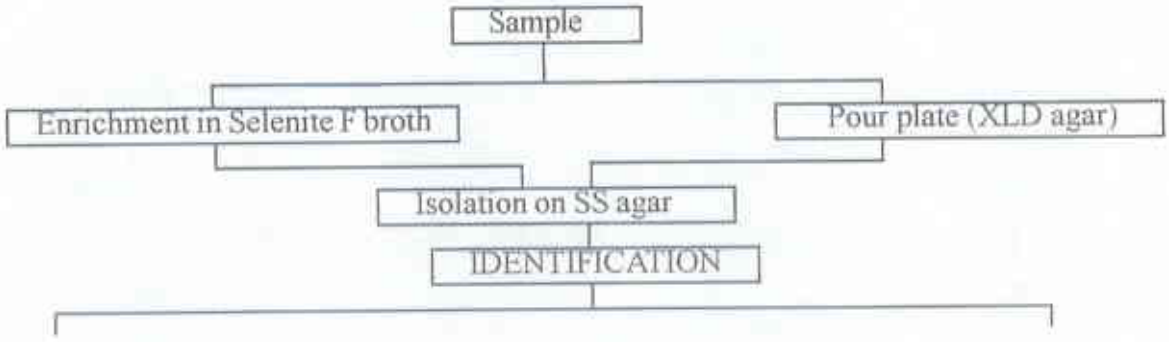
Cultural Character	Morphology	Biochemical Tests	
(a) Pink to dark-red colonies with metallic surface sheen on M-Endo agar	Gm-ve, nonsporing, noncapsulated bacilli	Catalase	+
		Oxidase	-
		Glucose (gas)	+
		Lactose (acid)	+
(b) Yellow colonies on XLD agar (Lactose fermenting)		Nitrate reduction	+
		Urease production	-
		Motility	+
		O-F	F
(c) Small colonies, dark, almost black centers, with greenish metallic sheen on EMB agar		Indole	+
		Methy Red	+
		Voges Proskauer	-
		Citrate	-
		H_2S production	-

GENERAL FLOW CHART FOR IDENTIFICATION OF ***Salmonella species***



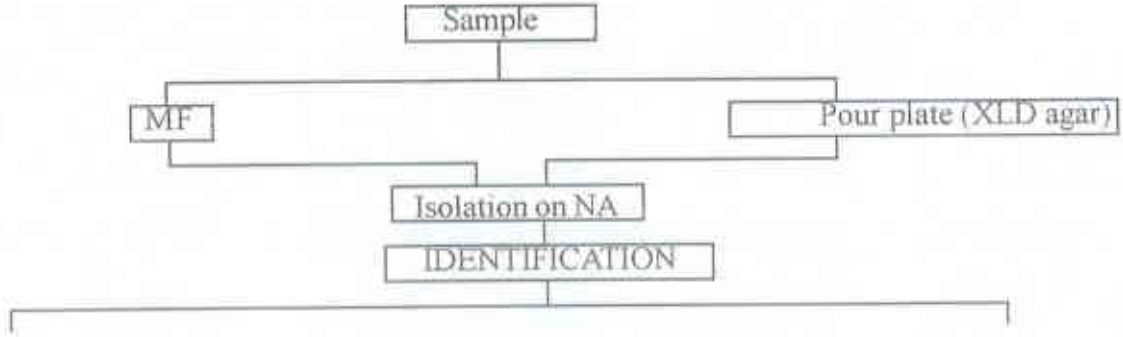
Cultural Character	Morphology	Biochemical Test	
(a) Red colonies with or without black centers on XLD agar	Gm-ve, nonsporing, noncapsulated bacilli	Catalase	+
		Oxidase	-
		Glucose (gas)	+
		but *S.typhi*	-
		Glucose (acid)	+
		Lactose (acid)	-
		Sucrose (acid)	-
(b) Colourless colonies with or without black centers on SS agar		Indole	-
		Methyl Red	+
		Voges-Proskauer	-
		Citrate ulilization	+
		H_2S production	+/-
		$N0_3$ reduction	+
		Urease	-
		Motility	+
		(rarely)	-
		O-F	F

GENERAL FLOW CHART FOR IDENTIFICATION OF ***Shigella species***



Cultural Character	Morphology	Biochemical Test	
(a) Red colonies without black centers on XLD agar	Gm-ve, nonsporing, noncapsulated	Catalase	+
		Oxidase	-
		Glucose (gas)	-
(b) Colourless colonies without black centers on SS agar	bacilli	rarely	+
		Glucose (acid)	+
		Lactose (acid)	-
		Sucrose (acid)	-
		Indole	-/+
		Methyl Red	+
		Voges - Proskauer	-
		Citrate Utilization	-
		H_2S production	-
		NO_3 reduction	+
		Urease production	-
		Motility	-
		O-F	F

GENERAL FLOW CHART FOR IDENTIFICATION OF ***Klebsiella species***



Cultural Character	Morphology	Biochemical Tests	
(a) Yellow colonies on XLD agar	Gm-ve, Nonsporing, Capsulated bacilli	Catalase	+
		Oxidase	-
(b) Large, raised, moist and mucoid colonies on NA		Glucose (gas)	v
		Lactose (acid)	v
		Sucorse (acid)	v
		Indole	-
		Methyl Red	v
		Voges - Proskauer	v
		Citrate utilization	v
		H_2S production	-
		$N0_3$ reduction	+
		Urease production	v
		Motility	-
		O-F	F

V= Variable

3.7 Study of Physico-Chemical Parameters of Water Samples

"Standard Methods for the Examination of Water and Wastewater" (APHA, 1995) was followed for the study of most of the physico-chemical parameters of water. The appearance, temperature and pH of water samples were recorded in the field during sampling period. All other parameters were analyzed in the Research Laboratory of the Department and in the Central Laboratory of NWSC, located at Sundarighat in Kathmandu.

Appearance

The general physical appearance of water sample was recorded by the unaided eye.

Temperature

Temperature was determined with the help of a standard mercury thermometer graduated upto 50°C. Soon after collection of the sample, a thermometer bulb was immersed into the water and noted the reading.

pH

Hydrogen ion concentration in the sample was measured with the help of a pH meter model-pH 8' (Yakogava Electric Corporation) by inserting the electrode into the water sample.

Conductivity

Conductivity was measured with the help of a conductivity meter. The electrode was first rinsed or washed with distilled water and then immersed into water sample, gently stirred and noted the reading.

Turbidity

The turbidity of a sample was measured with the help of a turbidimeter. The sample was put in the clean, free of any scratch Nephelometer sample tube taking a reference with standard turbidity suspension and noted the reading on the scale.

Total Acidity

100 ml of sample was taken in a conical flask and 2-3 drops of methyl orange indicator was added. The contents in the flask was stirred and a few drops of the phenolphthalein indica-

tor was added to the same sample. It was stirred again and then titrated with NaOH (0.05N) until the contents turned pink (pH 8.3). The volume of Na0H used in titrating the sample upto pH 8.3 was noted and total acidity was calculated by the following equation (Trivedy and Goel, 1986).

$$\text{Total Acidity, mg/l as } CaCO_3 = \frac{(A+B) \times N \text{ of Na0H} \times 1000 \times 50}{\text{ml sample}}$$

Where; A = Volume of Na0H used with methyl orange in titrating the sample to p^H 3.7

B = Volume of Na0H used with phenolphthalein in titrating the sample from p^H 3.7 to pH 8.3

N = Normality of standard alkali solution.

Total Alkalinity

For this, 100 ml of sample was taken in a conical flask and 2 drops of phenophthalein indicator was added. In all the cases, the solution remained colourless, that is, phenolphthalein alkalinity (PA) was absent. Then 2-3 drops of methyl orange was added to the same sample and titrated with HCl (0.1N) until the yellow colour changed to pink at end-point. The volume of HCl used in titrating the sample was noted and total alkalinity was calculated by the following equation (APHA, 1995).

$$\text{Total Alkalinity, mg/l as } CaCO_3 = \frac{A \times N \times 1000 \times 50}{\text{ml of sample}}$$

where; A = Volume of total HCl used with phenolphthalein and methyl orange indicators

N= Normality of standard acid

Total Hardness

For this, 50 ml of sample was taken in a clean conical flask to which 1 ml of ammonia buffer solution was added and stirred for thorough mixing. Then 200 mg of Eriochrome Black T indicator was added and shaken well. The contents in the flask was titrated against standard EDTA solution (0.01M) with continuous stirring, until the colour changed from wine red to blue at the end point. The volume of EDTA consumed was noted and total hardness was calculated by following equation (APHA, 1995).

$$\text{Total hardness, mg/l as } CaC0_3 = \frac{\text{ml EDTA used} \times 1000}{\text{ml sample}}$$

Calcium

For this, 50 ml sample was taken in a conical flask to which 2 ml of Na0H(1N) was added and stirred well. Then 200 mg of murexide (ammonium purpurate) indicator was added to the solution and titrated slowly against standard EDTA solution (0.01M) with continuous stirring, until the pink colour changed to purple at the end point. The volume of titrant consumed was noted and calcium in the water was calculated by using following equation (APHA, 1995).

$$\text{Calcium, mg/l} = \frac{A \times 400.8}{\text{ml sample}}$$

Where; A = volume of EDTA used.

Calcium Hardness

Calcium hardness is calculated by the following relation (APHA, 1995).

$$\text{Calcium Hardness, mg/l as } CaC0_3 = \frac{A \times 1000}{\text{ml sample}}$$

Where; A = Volume of EDTA consumed in calcium estimation

Magnesium

Magnesium was estimated by calculation method as follows (APHA, 1995.).

Magnesium, mg/l = [Total Hardness (as mg $CaC0_3$/l) - Calcium Hardness (as mg $CaC0_3$/l)] x 0.243]

Magnesium Hardness

Magnesium hardness is calculated by calculation method using following relation :

Magnesium hardness, mg/l = Total Hardness - Ca Hardness

Chloride

For chloride, 50 ml sample was taken in a conical flask and 2 ml of K_2Cr0_4 solution (5%) was added to it. The solution was titrated against $AgN0_3$ (0.02N) until a persistent red tinge appears. The volume of $AgN0_3$ consumed was noted and chloride in the water sample was estimated by using following equation (Trivedy and Goel, 1986).

$$\text{Chloride, mg/l} = \frac{A \times N \times 1000 \times 35.5}{\text{ml sample}}$$

where; A = ml of titrant consumed

N = normality of $AgN0_3$ solution

Iron

Iron contained in the water sample was determined by Phenanthroline method as described in APHA, 1995. For this, 50 ml sample was taken in 100 ml conical flask to which 2 ml conc. HCl and 1 ml of hydroxylamine hydrochloride ($NH_20H.HCl$) solution were added with separate clean pipettes. The contents in the flask was boiled to half of the volume for dissolution of all the iron and then cooled to room temperature and transferred to a 100-ml volumetric flask. Then, 10 ml ammonium acetate (CH_3C00NH_4) buffer solution and 4ml phenanthroline solution were added when orange-red colour was developed. It was diluted to 100 ml with distilled water, mixed thoroughly and allowed to stand at least 10 to 15 minute for maximum colour development. The reading was taken at 510 nm on a spectrophotometer treating distilled water as a blank. The concentration of total iron in the sample was directly calculated from the calibration curve prepared by using various dilutions of standard iron solution (1 to 4mg/l).

Nitrogen - Ammonia

Nitrogen-ammonia was determined as described in APHA, 1989. One ml of $ZnSO_4$ was added to 100ml of sample taken in a conical flask. The pH of the contents of flask was made 10.5 using 6N NAOH and left for few minutes. The sample was filtered discarding first 25ml. One drop of EDTA reagent was added to 50ml of filtrate collected, in case of high concentrations of calcium, magnesium ions, otherwise 2ml of Nessler's reagent was added. It was left for 10mins. and then colour developed was measured at 450 nm, and concentration of ammonia

was determined using standard calibration curve.

Calibration of Standard Curve

(a) 0.2, 0.7, 1.4,2.0,3.0, 4.0 and 5.0ml of standard NH_4Cl were taken in separate beakers.

(b) To all beakers, 2ml of Nessler's reagent was added and left for 10mins.

(c) Absorbance of individual beaker content was read at 450 nm.

(d) Standard curve was obtained by plotting a graph of absorbance against concentrations.

Dissolved Oxygen (DO)

Dissolved oxygen level in the water sample was calculated by the Winkler or iodometric method. Water sample was filled in BOD bottle of 300 ml capacity. A care was taken to exclude air bubbles during placing the stopper on the bottle. The stopper was removed and added 2ml $MnS0_4$ solution, followed by 2ml alkaline KI solution to the bottle with separate pipettes. Then, the stopper was carefully replaced excluding the trapping of air bubbles and mixed by inverting the bottle few times. The bottle was left to settle down the precipitate, and thus the dissolved oxygen in the sample was fixed in the field during sampling period.

The sample bottle was transported to the laboratory. When precipitate has settled sufficiently to leave clear supernate above the manganese hydroxide floc, 1-2 ml conc. H_2SO_4 was added to the bottle. Again the bottle was restopperd and mixed by inverting several times until the dissolution of precipitate was complete. Then, 100 ml of sample from BOD bottle was taken in a conical flask and titrated against 0.025 M $Na_2S_20_3$ solution to a pale straw colour. A few drops of starch solution was added and titration was continued until the blue colour disappeared. The volume of titrant consumed in getting the end point was noted. Three readings were taken and mean was taken which was converted into DO value by following relation (APHA, 1995).

$$DO, mg/l = \frac{A \times N \times 8 \times 1000}{\frac{V_2 (V_1 - V)}{V_1}}$$

Where; A = ml of titrant consumed

N = normality of $Na_2S_20_3$ solution

V = Volume of $MnS0_4$ and Alkaline KI used

V_1 = Capacity of BOD bottle after placing the stopper

V_2 = Volume of the part of the contents titrated

Biochemical Oxygen Demand (BOD)

For the determination of BOD, water sample was filled in clean and dry BOD bottle of 300 ml capacity. A care was taken not to trap any air bubble in the bottle during placing the stopper. The bottle was wrapped with paper and transported to the laboratory as soon as possible, and incubated at 20°C for 5 days in the BOD incubator, DO remained in the water sample after 5 days incubation at 20°C was determined by Winkler's iodometric method as described in DO. This DO was DO_5 of the water sample and DO determined on the initial day was DO_1 of the sample. BOD was calculated by using following equation (APHA, 1995).

$$BOD, mg/l = DO_1 - DO_5$$

Where; DO_1 = Initial DO in the sample

DO_5 = DO after 5 days

3.8 Study of Antibiotic Sensitivity and Resistance of Isolates

Antibiotic sensitivity or resistance of isolated enteric bacteria was assayed using a modified Kirby-Bauer disk-diffusion method (Bauer *et al.*, 1966). Cells were grown at 37°C in 5 ml of nutrient broth for about 4 hrs. using pure cultures as inoculum. The turbidity developed was compared with that of standard barium sulphate. A sterile cotton swab was dipped into the properly prepared inoculum and firmly rotated against the upper inside wall of the tube to express excess fluid, and then swabbed onto Mueller-Hinton agar. During swabbing the plate was streaked with the swab three times turning the plate 60° between each streaking to achieve a lawn of confluent bacterial growth. The plate was kept at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculum. Sensitivity discs from their respective vials were carefully placed in the plate with the help of a flamed forceps, at equal distance and sufficiently separated from each other to avoid the overlapping of the zones of inhibition. The discs were lightly pressed with the forceps to make complete contact with the surface of the medium. The plate was allowed to stand at room temperature for 30 minutes for prediffusion and then incubated at 37°C for 16 to 18 hrs.. The diameter of the zone of inhibition was measured at the end of the incubation period. Organisms were classified as sensitive or resistant to an

antibiotic according to the diameter of the inhibition zone surrounding each antibiotic disk as listed by the manufacturer. Organisms considered to be of intermediate resistance were scored as sensitive.

Antibiotics tested and standard disk concentrations were as follows :

Amikacin	(Ak), 30 mcg	Co-Trimoxazole	(Co), 25 mcg
Gentamicin	(G), 10 mcg	Nalidix Acid	(Na), 30 mcg
Nitrofurantoin	(Nf), 300 mcg	Tetracycline	(T), 30 mcg
Ampicillin	(A), 10 mcg	Ceftazidime	(Ca), 30 mcg
Chloramphenicol	(C), 30 mcg	Ciprofloxacin	(Cf), 5 mcg

Preparation of Barium Sulphate Standard

- 99.5 ml of H_2SO_4 (0.36N) was taken in a clean and dry test tube.
- 0.5 ml of $BaCl_2.2H_2O$ solution (1.175%) was added to it and the tube was shaken to mix the contents well.

3.9 Study of Oligodynamic Action Against Some Enteric Bacteria Isolates

The study of inhibitory effect of metals against test organisms was done as described by Benson, 1979. Nutrient agar (NA) was liquefied, cooled to 50°C and inoculated with broth culture of test organism. About half of the medium was poured into a sterile petriplate and the other half was left in water bath at 50°C. Five metallic disks (Copper, silver, aluminium, brass and stainless steel) were carefully placed on the solidified agar surface in the plate at equal distance and sufficiently separated from each other with the help of a flamed forceps. The metallic disks before placing on the agar were cleaned, one at a time, as follows :

- washed first with soap and water; then rinsed with water.
- with flamed forceps dipped in acid-alcohol and rinsed with distilled water.

The remaining seeded agar was poured from the conical flask over the metal disks, and then the plate was incubated for 48 hrs. at 37°C. The zone of inhibition for each disk was measured at the end of the incubation period.

CHAPTER- FOUR

RESULTS

This chapter describes the existing water quality of the traditional groundwater supplies used for drinking in urban Patan city.

4.1 Bacteriological Quality

4.1.1 Total and Faecal Coliform Count

Out of 70 groundwater sources, 60(85.6%) showed the presence of total coliforms, while faecal coliforms were detected in 48(68.6%) at the time of study (Table 9). In this study, 48(68.6%) of the total samples were found to exceed the WHO permissible level containing total coliforms (> 10 CFU/100ml) and faecal coliforms (Table 10).

Table 4 shows that all shallow pump water samples (100%) were not contaminated with indicator organisms during the study period. Shallow well water samples (100%) exhibited high recovery of total coliforms (160 to 880 CFU/100ml) and faecal coliforms (40 to 800 CFU/ 100ml) as shown in Table 5.

Water samples from 2 (14.3%) stone spouts only showed absence of indicator organisms, while rest 12 (85.7%) were out of WHO permissible limit having total and faecal coliform counts 25 to 7840 CFU/100ml and 21 to 3660 CFU/100ml , respectively (Table 6).

The results (Table 7) of protected well water samples indicated the quality of water to be variable. Sample from 1 (5%) source showed zero coliform bacteria, 9(45%) and 10(50%) sources exhibited the count within and out of WHO permissible value, respectively. In overall 10 (50%) sources were acceptable, while rest 10 (50%) were unacceptable in regard to bacteriological quality. Total and faecal coliform densities ranged from 2 to 290 CFU/100ml and 1 to 280 CFU/ 100ml respectively.

Results presented in Table 8 clearly indicates that most of the unprotected dug wells were contaminated with faecal material. Total and faecal coliform counts were mostly above

the WHO permissible level. Only 2(7.2%) sources were free of indicator organisms, while 3 (10.7%) and 23 (82.1%) lie within and out of WHO permissible value. In overall, 5 (17.9%) and 23 (82.1%) sources were found acceptable and unsafe, respectively during the study period in regard to bacteriological quality. Total and faecal coliform densities ranged from 10 to 960 CFU/100ml and 2 to 520 CFU/100ml, respectively.

4.2 Isolation and Identification of Isolated Enterobacteria

In this study 120 enteric bacteria were isolated from 49 out of 70 various groundwater sources. The isolated organisms were subjected to various biochemical tests for their identification. The results of the features used in the identification, and the identified organisms are given in Appendix-I.

The organisms identified include *Escherichia sp.*, *Enterobacter sp.*, *Citrobacter sp.*, *Salmonella sp.* *Shigella sp.*, *Serratia sp.*, *Yersinia sp.*, *Hafnia alvei* and others. Recovery of *Enterobacter sp.* was found to be maximum followed by *Escherichia sp.*, *Citrobacter sp.*, and *Salmonella sp.*, sequentially, and others. Percentage recovery of different enterobacteria from various groundwater sources are shown in Table 11.

Maximum enterobacteria isolates (45.9%), particularly *Enterobacter sp.* (15.8%) and *Escherichia sp.* (9.2%), were recovered from unprotected wells. Stone spout and protected well samples also exhibited high recovery of enteric bacteria, but the recovery was nil from shallow pump samples and minimum from shallow wells.

In overall, 70% of the total sources exhibited different enterobacteria pointing out to the necessity of proper treatment disposal and management of wastes.

Table 4 : Bacteriological Analysis of Shallow Pump Water Samples.

S.N.	Sample Code (Sources)	Total Coliform Count CFU/100ml	Faecal Coliform Count CFU/100ml	WHO Permissible Value
1.	SP1	0	0	Coliform Organisms 10 CFU/100ml Faecal Coliforms 0 CFU/100ml
2.	SP2	0	0	
3.	SP3	0	0	
4.	SP4	0	0	
5.	SP5	0	0	

SP = Shallow Pump

Table 5 : Bacteriological Analysis of Shallow Well Water Samples.

S.N.	Sample Code (Sources)	Total Coliform Count CFU/100ml	Faecal Coliform Count CFU/100ml
1.	SW1	448	416
2.	SW2	160	40
3.	SW3	880	800

SW = Shallow Well

Table 6 : Bacteriological Analysis of Stone Spout Water Samples.

S.N.	Samples Code (Sources)	Total Coliform Count CFU/100ml	Faecal Coliform Count CFU/100ml
1.	SS1	1160	900
2.	SS2	5120	3660
3.	SS3	960	600
4.	SS4	25	21
5.	SS5	0	0
6.	SS6	0	0
7.	SS7	1300	1140
8.	SS8	7840	1580
9.	SS9	3260	1960
10.	SS10	3400	1360
11.	SS11	120	100
12.	SS12	300	200
13.	SS13	280	90
14.	SS14	980	520

SS = Stone Spout

Table 7 : Bacteriological Analysis of Protected Well Water Samples.

S.N.	Sample Code (Sources)	Total Coliform Count CFU/100ml	Faecal Coliform Count CFU/100ml	WHO Permissible Value
1.	WC1	2	0	Coliform Organisms 10 CFU/100ml Faecal Coliforms 0 CFU/100ml
2.	WC2	170	10	
3.	WC3	220	180	
4.	WC4	2	0	
5.	WC5	20	1	
6.	WC6	2	0	
7.	WC7	290	280	
8.	WC8	20	2	
9.	WC9	10	0	
10.	WC10	4	0	
11.	WC11	2	0	
12.	WC12	2	0	
13.	WC13	26	2	
14.	WC14	0	0	
15.	WC15	20	1	
16.	WC16	57	40	
17.	WC17	90	18	
18.	WC18	210	130	
19.	WC19	4	0	
20	WC20	2	0	

WC = Protected Well

Table 8 : Bacteriological Analysis of Unprotected Well Water Samples.

S.N.	Sample Code (Sources)	Total Coliform Count CFU/100ml	Faecal Coliform Count CFU/100ml
1.	WO1	200	60
2.	WO2	360	40
3.	WO3	70	20
4.	WO4	80	40
5.	WO5	50	20
6.	WO6	43	40
7.	WO7	20	4
8.	WO8	110	40
9.	WO9	30	10
10.	WO10	0	0
11.	WO11	440	160
12.	WO12	220	80
13.	WO13	240	160
14.	WO14	10	0
15.	WO15	0	0
16.	WO16	10	0
17.	WO17	400	320
18.	WO18	60	10
19.	WO19	20	4
20.	WO20	290	180
21.	WO21	120	20
22.	WO22	960	40
23.	WO23	20	4
24.	WO24	120	80
25.	WO25	30	2
26.	WO26	120	80
27.	WO27	10	0
28.	WO28	720	520

WO = Unprotected Well

Fig 1: Total and Faecal Coliform Densities Recovered from Shallow Well Water Samples

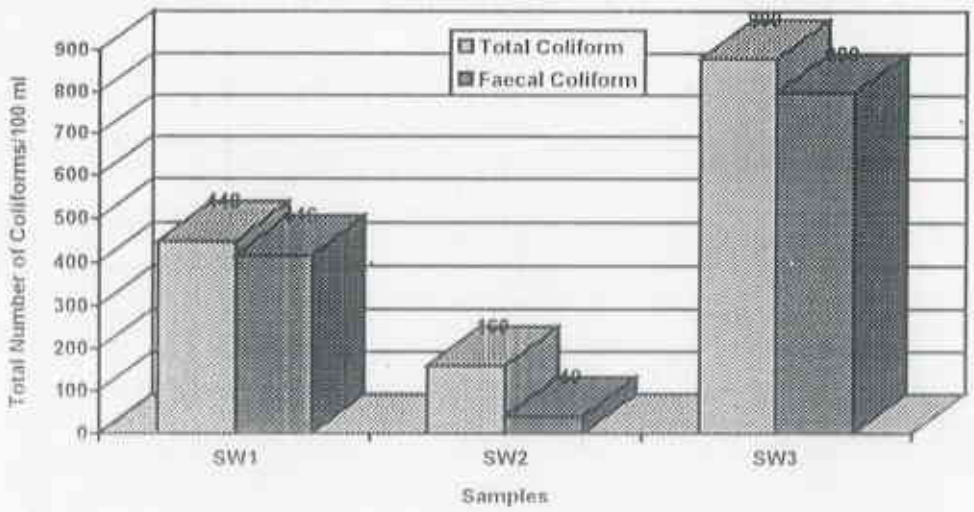


Fig 2: Total and Faecal Coliform Densities Recovered from Stone Spout Water Samples

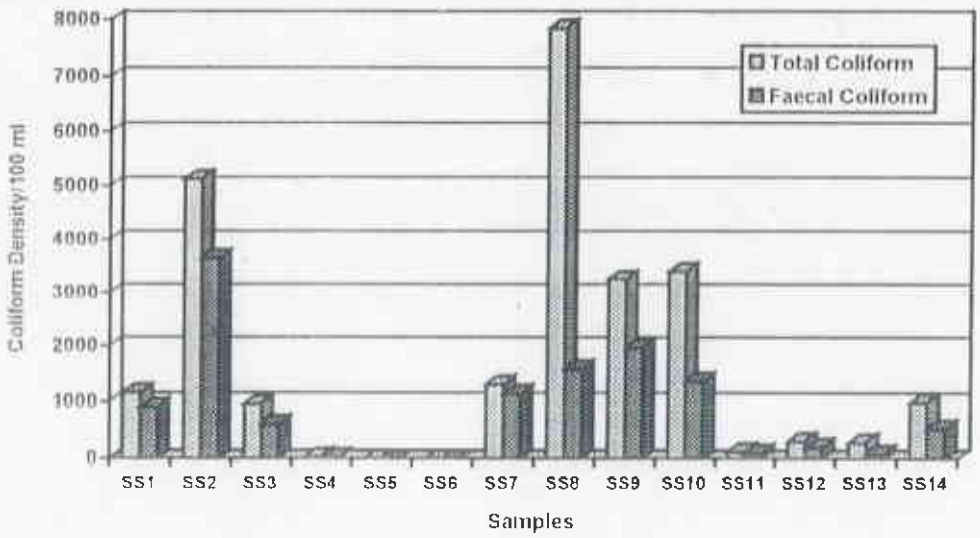


Fig 3: Total and Faecal Coliform Densities Recovered from Protected Well Water Samples

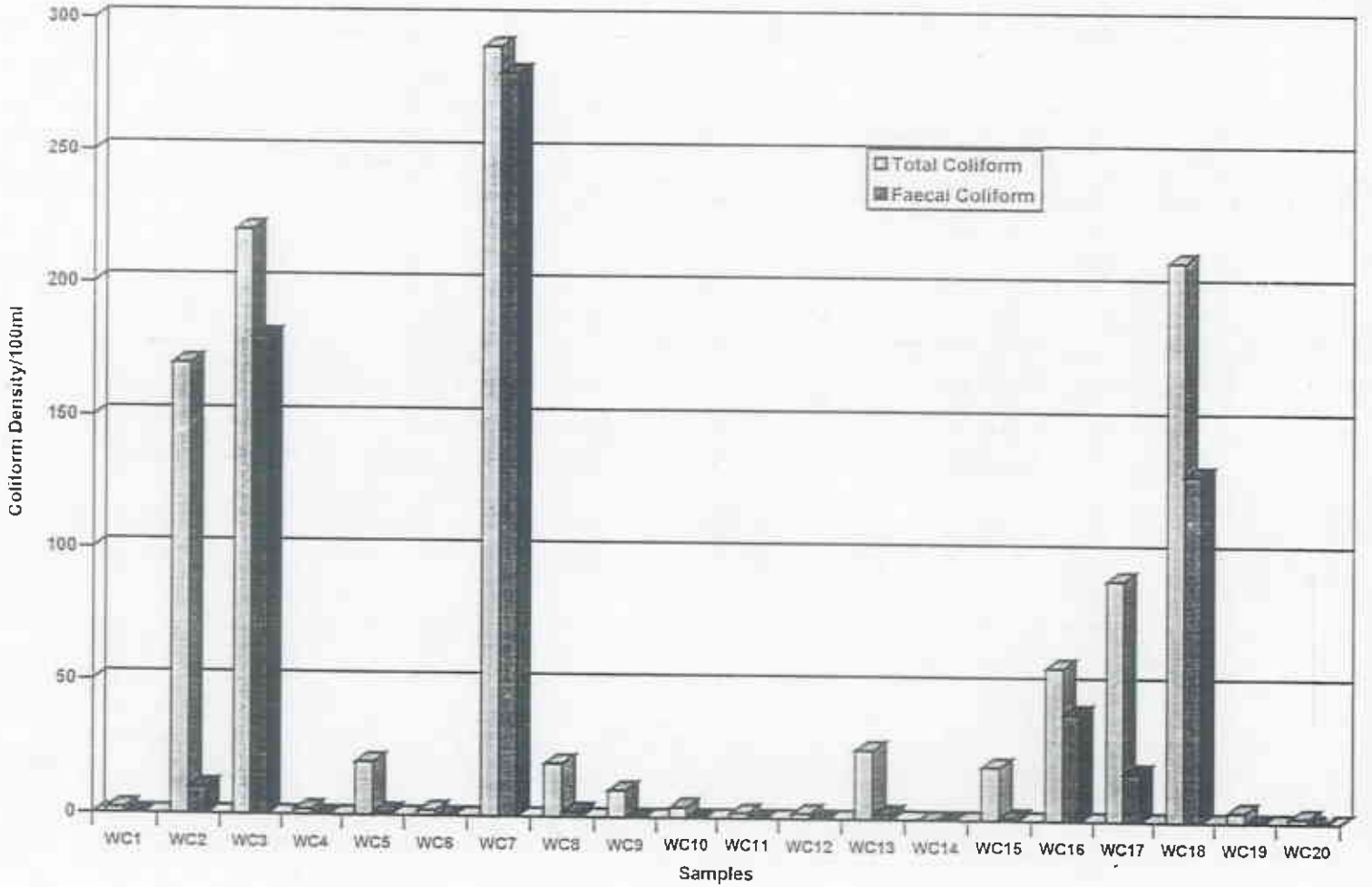


Fig.4: Total and Faecal Coliform Densities Recovered from Unprotected Well Water Samples

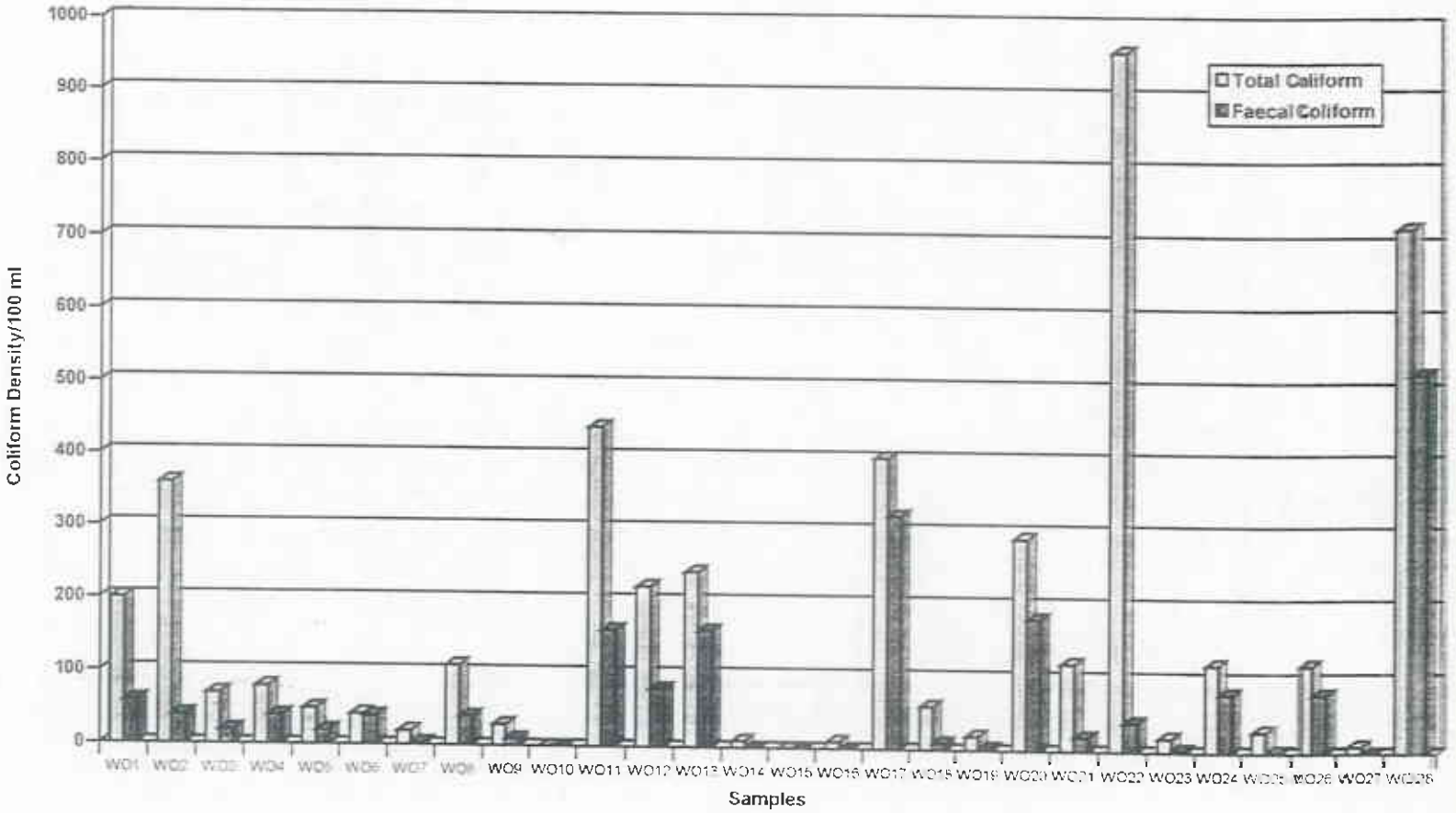


Plate 1 : Colonies of Total Coliforms on M-Endo agar

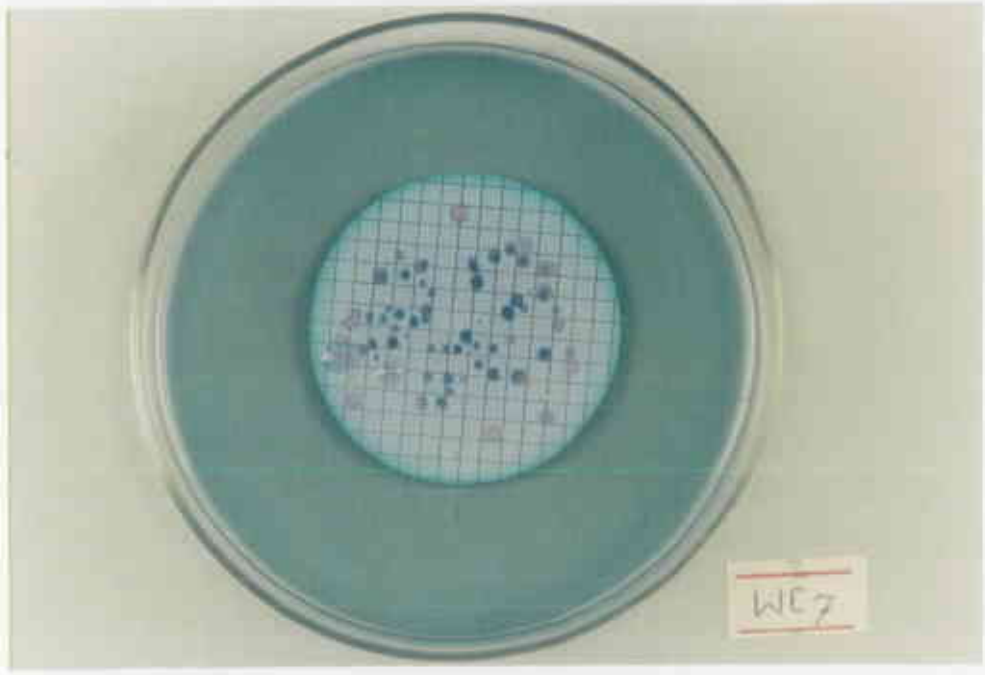


Plate 2 : Colonies of Faecal Coliforms on M-FC agar

Table 9 : Percentage of Various Groundwater Sources Showing Total and Faecal Coliforms

Coliforms	Shallow Pump	Shallow Well	Stone Spout	Protected Well	Unprotected Well	Total
Total Coliforms	-	3 (4.3)	12 (17.1)	19 (27.1)	26 (37.1)	60 (85.6)
Faecal Coliforms	-	3 (4.3)	12 (17.1)	10 (14.3)	23 (32.9)	48 (68.6)

Figures in the Parentheses are sources in Percentage with coliforms.

Table 10 : Percentage of Various Groundwater Sources Showing Bacterial Count Within and Exceeding WHO Guideline Value

S.N.	Sources	Number of Sources Showing		Total Number of Sources	
		Total Coliforms > 10 col/100ml	Faecal Coliforms <1 col/100ml	Within WHO Standard	Exceeding WHC Standard
1.	Shallow Pump	-	5 (7.1)	22 (31.4)	48 (68.6)
2.	Shallow Well	3 (4.3)	-		
3.	Stone Spout	12 (17.1)	2 (2.9)		
4.	Protected Well	10 (14.3)	10 (14.3)		
5.	Unprotected Well	23 (32.9)	5 (7.1)		

Figures in the Parentheses are sources in Percentage.

Table 11: Percentage Distribution of Different Enterobacteria in Various Groundwater Sources in Urban Area of Patan City

Enterobacteria	Shallow Pump	Shallow Well	Stone Spout	Protected Well	Unprotected Well	Total
Enterobacter sp.	-	3 (2.5)	8 (6.7)	6 (5.0)	19 (15.8)	36 (30.0)
Escherichia sp.	-	3 (2.5)	7 (5.8)	4 (3.3)	11 (9.2)	25 (20.8)
Citrobacter sp.	-	1 (0.8)	4 (3.3)	2 (1.7)	8 (6.7)	15 (12.5)
Salmonella sp.	-	-	4 (3.3)	4 (3.3)	5 (4.2)	13 (10.8)
Shigella sp.	-	-	3 (2.5)	-	3 (2.5)	6 (5.0)
Klebsiella sp.	-	-	1 (0.9)	1 (0.8)	-	2 (1.7)
Others	-	-	9 (7.5)	5 (4.2)	9 (7.5)	23 (19.2)
Total	-	7 (5.8)	36 (30.0)	22 (18.3)	55 (45.9)	120 (100.00)

Figures in the Parentheses are Percent Recovery of Enterobacteria.

Fig 5: Various Groundwater Sources Within and Exceeding WHO Guideline Value in Regard to Coliform Densities

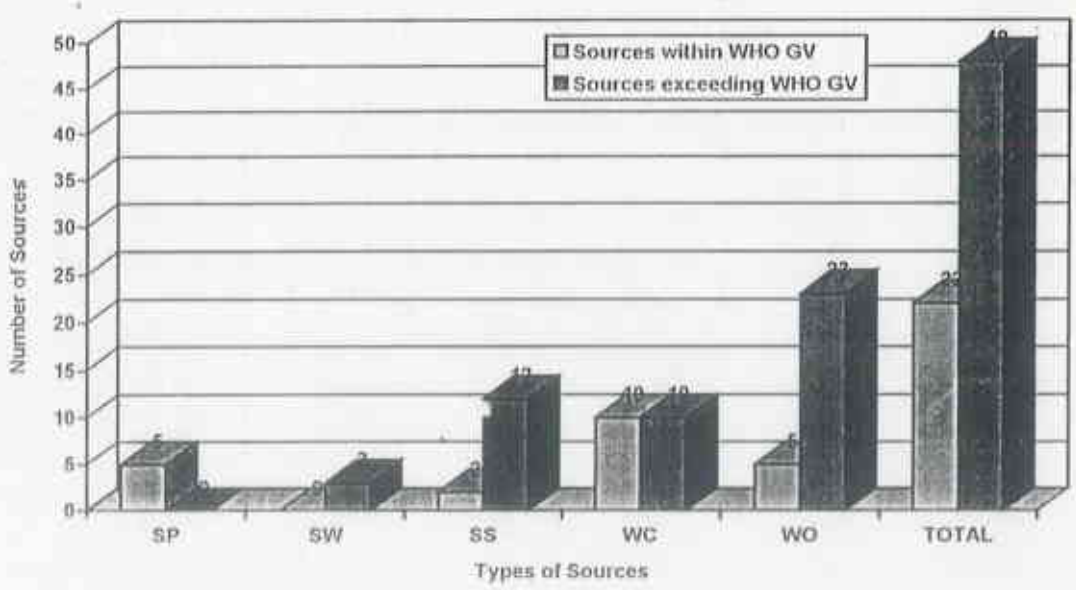
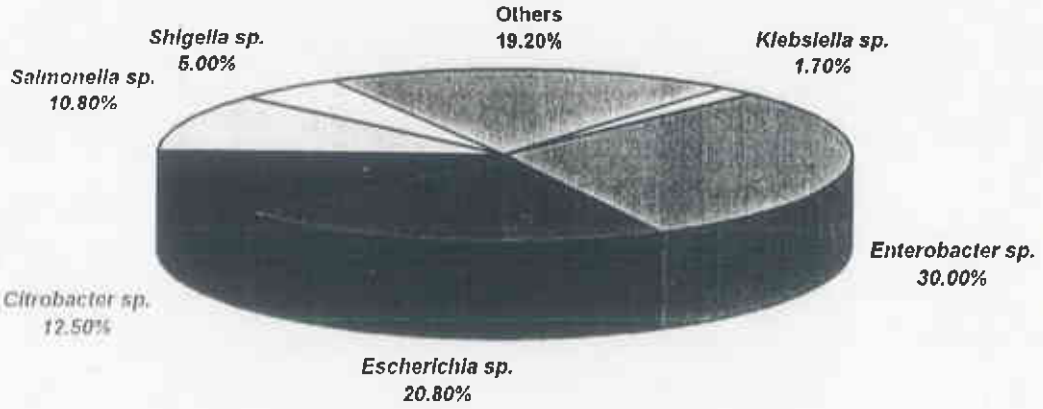


Fig.6: Enterobacteria Isolates in Percentage.



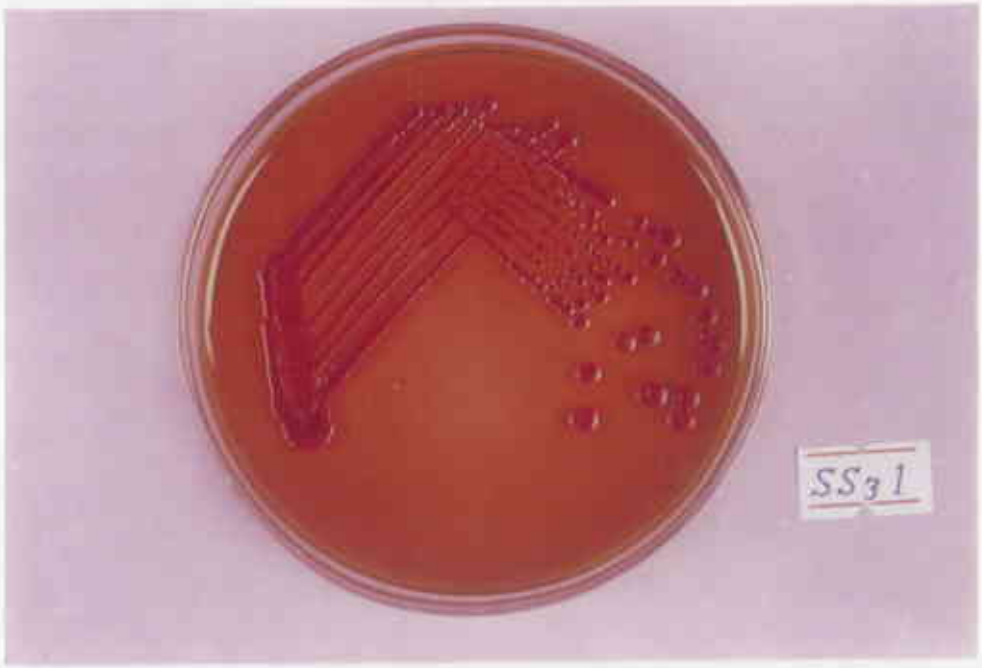


Plate 3 : Colonies of *E.coli* on Eosin Methylene Blue (EMB) agar

Plate 4 : Colonies of *Salmonella sp.* (Left) and *E. coli* (Right) on Xylose Lysine Deoxycholate (XLD) agar

Plate 5 : Colonies of *Salmonella sp.* (Left) and *Shigella sp.* (Right) on Salmonella-Shigella (S-S) agar

Plate 6 : Deteriorating Condition of a Dug Well in Urban Patan

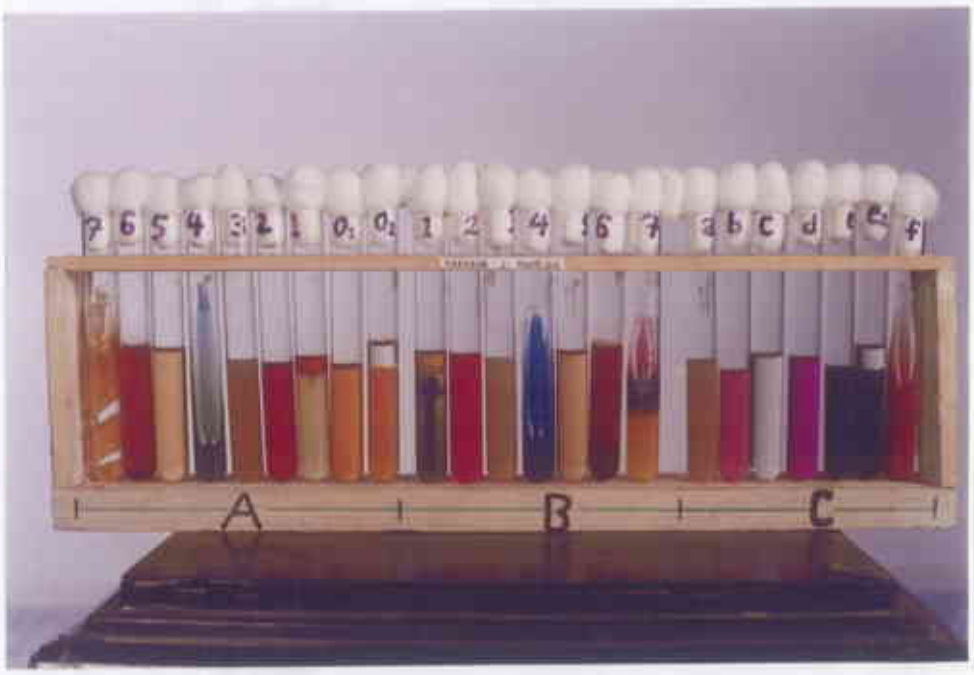


Plate 7 : Tubes Showing Results of Biochemical Tests for *E. coli* (Set A) and *Salmonella sp.*(Set B). Set C - Control Tubes.

Set A and Set B		Set C
Code	Tests	Code
O_1 and O_2	Aerobic or anaerobic utilization of carbohydrates	e_1 and e_2
1	H_2S and Indole production, and Motility	
2	Methyl Red	a (-ve)
3	Voges-Proskauer	b (+ve)
4	Citrate utilization	
5	Urease production	d (+ve)
6	Nitrate reduction	c (-ve)
7	Fermentation of carbohydrates, H_2S and Gas production	f (Before Incubation)

4.3 Physico-Chemical Analysis of Water Samples

The physical and chemical parameters of water samples analyzed from various groundwater sources in urban Patan city are given in Tables 12,13, 14, 15 and 16.

Almost all water samples were clear in appearance, except very few at the time of study. The temperature of water samples ranged from 19.1 to 22.0°C, 19.2 to 20.5°C, 19.0 to 25°C, 18.8 to 22.0°C and 17.5 to 21.4°C for shallow pumps, shallow wells, stone spouts, protected wells and unprotected wells, respectively. The minimum and maximum temperatures recorded were 17.5°C and 25.0°C from unprotected well (W03) and stone spout (SS1), respectively. Most of the sources (88.6%) were recorded within permissible limit of pH value while some (11.4%) were found with pH values slightly less than the WHO guideline value. The pH values for water samples ranged from 6.16 (WC 18) to 7.76 (WC 10). The turbidity values from 61 (87.1%) sources lie within the WHO recommended value (5 NTU), while rest 9 (12.9%) sources exceeded the permissible value. However, only one source (WC4) was found out of permissible value (5-25 NTU) proposed by NWSC. The maximum and minimum values recorded were 90 NTU and 0.4 NTU, respectively.

Similarly, conductivity values for almost all water samples (94.3%) were recorded within the maximum allowable limit (1,250 μS/cm) proposed by NWSC. The values for conductivity ranged from 248 μS/cm (W022) to 1726 μS/cm (W03). The values for total alkalinity and acidity ranged from 75mg/l (WC16) to 425 mg/l (W018), and 5mg/l (WC10) to 175 mg/l (SP1), respectively.

Almost all water samples (95.7%) were found within the acceptable limit (100-500mg/l) proposed by NWSC, and WHO guideline value for hardness as well. Two samples (SP5 and SS11) were recorded with the values (70 mg/l and 92 mg/l respectively) less than the permissible limit and one sample (WC7, 548 mg/l) exceeded the maximum allowable limit. The values for total hardness ranged from 70 to 548 mg/l. Similarly levels of calcium hardness and magnesium hardness ranged from 24 mg/l (W01) to 212 mg/l (W018), and 12mg/l (SS3) to 468mg/l (WC7), respectively.

The calcium content of the water samples ranged from 18.44 mg/l (SP5) to 84.97 mg/l (W018). Only 5 (7.1%) sources were recorded within permissible limit (75-200 mg/l) proposed

by NWSC, while rest 65 (92.9%) showed fairly low values. Similarly, the concentrations of magnesium ranged from 3.88 mg/l (SW2) to 114.18mg/l (WC7) for various sources. Only 9 (12.9%) sources lie within the recommended value (30-150mg/l) proposed by NWSC, while rest 61 (87.1%) were found with the values less than the acceptable level.

The maximum level of total iron content recorded was 4.03mg/l (WC4), the minimum being 0.01 mg/l (16 sources). The result exhibits 14.3% of the sources to lie within the WHO guideline value (0.3 mg/l), 12.9% exceeded the safety limit and 72.8% with fairly low values than the permissible limit. In overall, 87.1% of the total sources were safe, while rest 12.9% were unsafe. However, if the standard of total iron in drinking water proposed by NWSC (0.1 to 1mg/l) is followed, then 22.9% and 4.3% of the samples were found to lie within and out of maximum allowable limit, while 72.8% samples showed fairly low values.

The concentration of total ammonia ranged from fairly low to extremely high exceeding the maximum allowable limit. The maximum ammonia concentration recorded was 96 mg/l and the minimum was 0.02 mg/l. Out of total 43 (61.4%) sources were found within the WHO permissible limit (1.5 mg/l), while rest 27 (38.6%) exceeded the allowable limit for ammonia content in drinking water. However, if the standard of total ammonia in drinking water proposed by NWSC (0.05 to 1.5mg/l) is followed, then 32 (45.7%) sources were within the permissible value, 27 (38.6%) exceeded the maximum allowable limit and 11 (15.7%) with the values less than the permissible level.

The chloride content of water samples ranged from 14.2 mg/l (SP5 and W022) to 284 mg/l (WC2). The chloride concentration exceeded the WHO recommended value (250 mg/l) for only one water sample (WC2).

Levels of DO ranged from 1.01 mg/l (WC4) to 8.90 mg/l (W024) during this study. Similarly, BOD values were found in a range of 0.20 mg/l to 1.81 mg/l (WO24). There is no WHO recommended value for DO and BOD in drinking water.

Table 12 : Physico-Chemical Analysis of Shallow Pump Water Samples

Parameters	Units	Sample Code (Sources)					WHO Guideline Value	Proposed Permissible Value (NWSC)
		SP1	SP2	SP3	SP4	SP5		
Appearance	-	Hazy	Hazy	Clear	Clear	Clear	-	-
Temperature	°C	22.0	20.8	20.6	20.0	19.1	-	-
pH	-	6.68	7.13	6.70	6.75	7.19	6.5-8.5	6.5-9.2
Turbidity	NTU	24.0	24.0	1.9	1.6	1.5	5	5-25
Conductiviy	µS/cm	811	1003	698	753	333	-	400-1250
Total Alkalinity	mg/l as $CaC0_3$	345	250	170	225	110	-	-
Total acidity	mg/l as $CaC0_3$	175	30	75	85	10	-	-
Total Hardness	mg/l as $CaC0_3$	296	220	196	224	70	500	100-500
Ca Hardness	mg/l as $CaC0_3$	152	132	140	144	46	-	-
Mg Hardness	mg/l as $CaC0_3$	144	88	56	80	24	-	-
Calcium	mg/l	60.92	52.91	56.11	57.72	18.44	-	75-200
Magnesium	mg/l	35.11	21.44	13.64	19.49	5.85	-	< 30-150
Total Iron	mg/l	1.91	0.50	0.16	0.08	0.03	0.3	0.1-1
Total Ammonia	mg/l	9.60	80.00	5.20	1.04	0.20	1.5	0.05-1.5
Chloride	mg/l	92.30	113.60	78.10	56.80	14.20	250	upto 250
DO	mg/l	1.22	1.82	1.21	2.43	4.25	-	-
BOD	mg/l	0.21	1.21	0.20	0.20	1.01	-	-

World Health Organisation (WHO) Guideline Value (1984).
NTU = Nephelometric Turbidity Unit
\- No health based value has been proposed
NWSC = Nepal Water Supply Corporation (Source : WHO, 1971)

Fig. 7: Graph Showing Iron and DO Values of Shallow Pump Water Samples

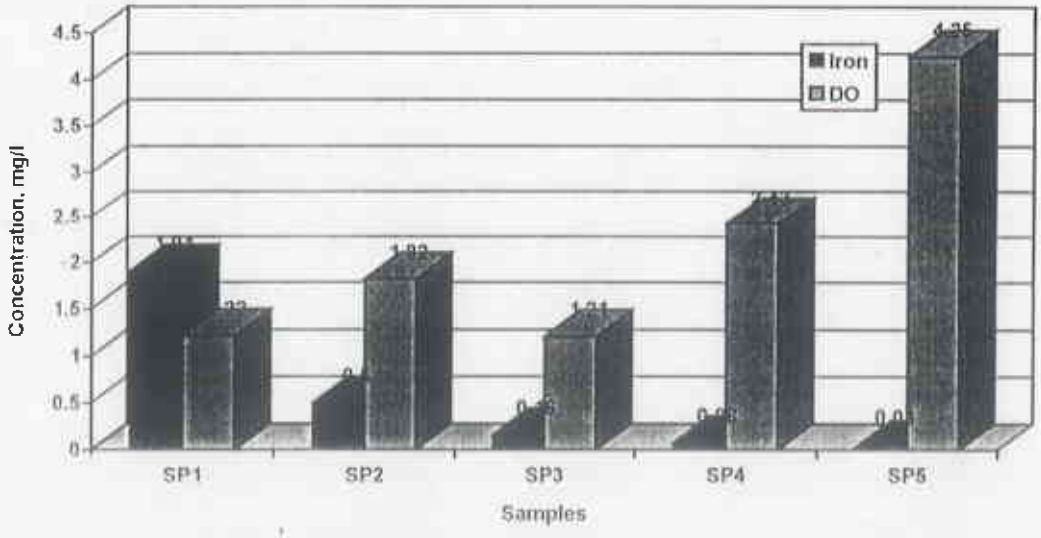


Fig. 8: Graph Showing Ammonia Values of Shallow Pump Water Samples

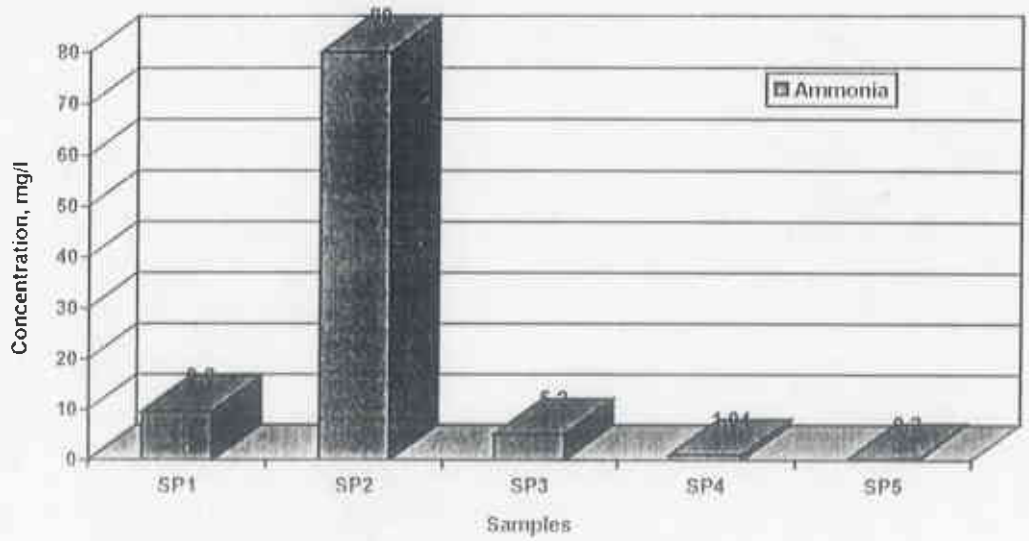


Table 13 : Physico-Chemical Analysis of Shallow Well Water Samples

Parameters	Units	Sample Code (Sources)			WHO Guideline Value	Proposed Permissible Value (NWSC)
		SW1	SW2	SW3		
Appearance	-	Clear	Clear	Clear	-	-
Temperature	°C	19.2	20.2	20.5	-	-
pH	-	6.59	7.06	6.77	6.5-8.5	6.5-9.2
Turbidity	NTU	0.4	2.0	3.1	5	5-25
Conductivity	µS/cm	523	452	854	-	400-1250
Total Alkalinity	mg/l as $CaC0_3$	95	155	170	-	-
Total Acidity	mg/l as $CaC0_3$	62.50	30.00	100.00	-	-
Total Hardness	mg/l as $CaC0_3$	168	116	288	500	100-500
Ca Hardness	mg/l as $CaC0_3$	108	100	168	-	-
Mg Hardness	mg/l as $CaC0_3$	60	16	120	-	-
Calcium	mg/l	43.29	40.08	67.33	-	75-200
Magnesium	mg/l	14.62	3.88	29.25	-	<30-150
Total Iron	mg/l	0.05	0.01	0.05	0.3	0.1-1
Total Ammonia	mg/l	1.04	2.00	16.00	1.5	0.05-1.5
Chloride	mg/l	65.32	42.60	92.30	250	upto 250
DO	mg/l	4.46	3.65	2.03	-	-
BOD	mg/l	1.01	0.81	0.41	-	-

Fig 9: Graph Showing Iron, Ammonia and DO Values of Shallow Well Water Samples

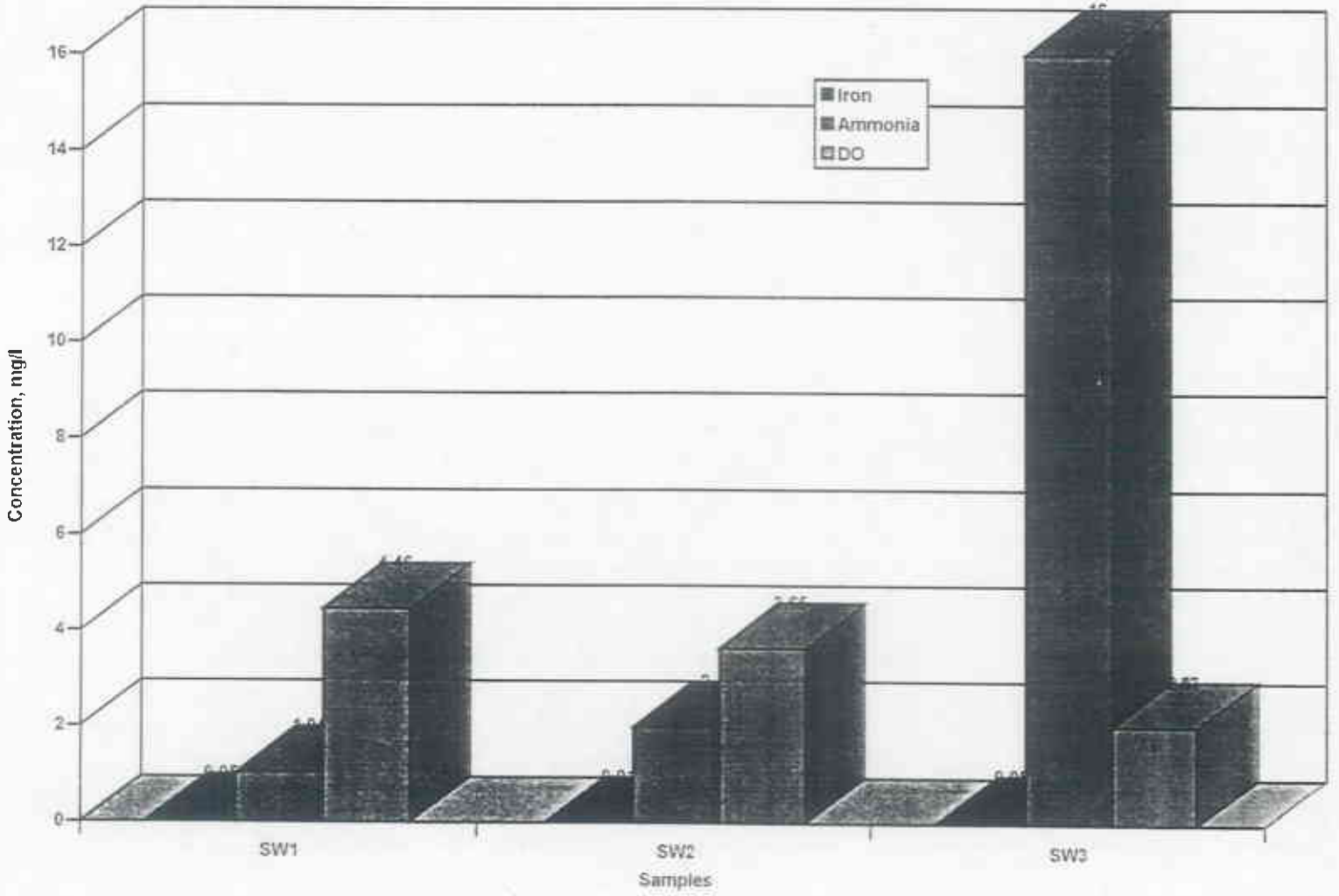


Table 14: Physico-Chemical Analysis of Stone Spout Water Samples.

Parameters	Units	Sample Code (Sources)													
		SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14
Appearance	-	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Temperature	°C	25.0	24.0	21.5	20.5	19.5	19.5	19.0	22.5	19.5	22.9	19.0	21.0	20.5	21.8
pH	-	6.41	6.33	7.02	6.55	6.74	6.81	6.81	6.28	6.83	6.83	6.29	6.77	6.95	6.53
Turbidity	NTU	0.5	0.5	0.8	1.3	0.4	1.1	2.9	1.2	0.4	1.6	0.5	0.4	10.0	0.5
Conductivity	µS/cm	589	546	1154	594	580	519	515	454	586	534	399	986	738	470
T.Alkalinity asCaC0$_3$	mg/l	140	105	240	140	90	90	105	90	130	280	95	165	200	95
T.Acidity asCaC0$_3$	mg/l	20	25	40	55	55	55	55	70	65	70	95	20	50	75
T.Hardness asCaC0$_3$	mg/l	160	124	204	156	144	148	140	118	164	172	92	196	172	118
Ca Hardness asCaC0$_3$	mg/l	104	84	192	92	80	104	88	84	96	152	60	160	132	90
Mg Hardness asCaC0$_3$	mg/l	56	40	12	64	64	44	52	34	68	20	32	36	40	28
Calcium	mg/l	41.68	33.67	76.95	36.87	32.06	41.68	35.27	33.67	38.48	60.92	24.05	64.13	52.91	36.07
Magnesium	mg/l	13.65	9.74	2.89	15.60	15.60	10.72	12.67	8.28	16.57	4.85	7.80	8.75	9.73	6.81
Total Iron	mg/l	0.05	0.01	0.05	0.03	0.01	0.01	0.01	0.01	0.01	0.05	0.01	0.01	2.97	0.01
Total Ammonia	mg/l	0.24	0.04	0.72	5.60	0.02	0.32	0.80	1.50	0.80	64.00	0.64	0.04	20.00	0.88
Chloride	mg/l	92.30	149.1	127.8	99.40	56.8	49.70	42.60	49.70	63.90	71.00	49.70	113.6	49.70	56.80
DO	mg/l	3.84	4.25	2.43	2.64	3.64	2.43	3.24	2.23	2.43	3.04	3.45	6.07	3.44	1.82
BOD	mg/l	0.81	0.39	0.71	0.61	0.61	0.20	0.81	1.42	0.40	0.61	0.21	0.60	1.41	0.40

Fig 10 Graph Showing Iron and DO Values of Stone Spout Water Samples.

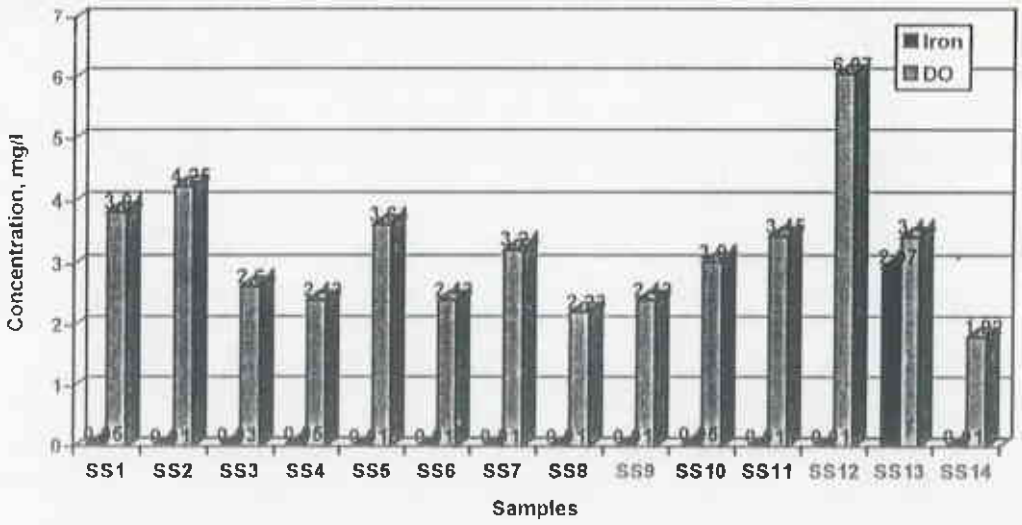


Fig 11: Graph Showing Ammonia Values of Stone Spout Water Samples.

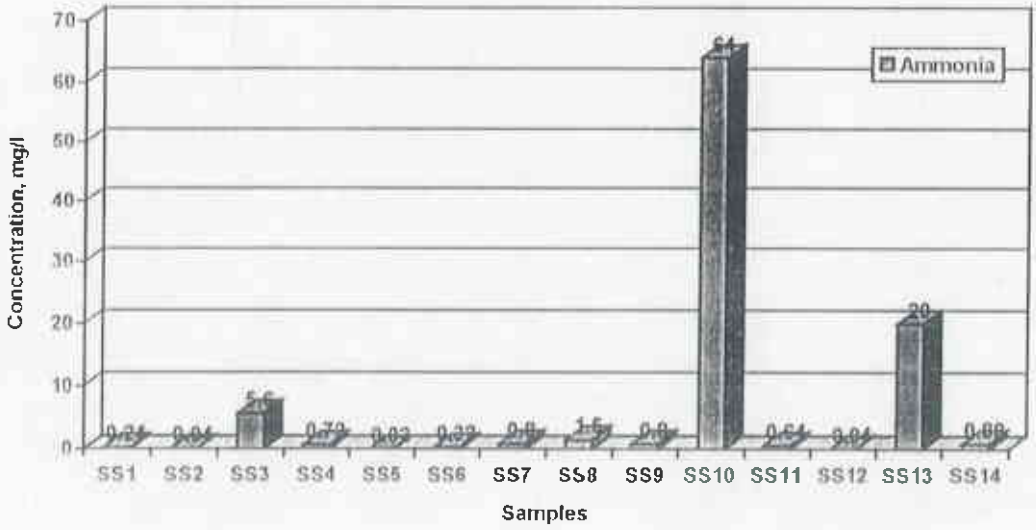


Table 15 (a) : Physico-Chemical Analysis of Protected Well Water Samples.

Parameters	Units	Sample Code (Sources)									
		WC1	WC2	WC3	WC4	WC5	WC6	WC7	WC8	WC9	WC10
Appearance	-	Clear	Clear	Clear	Hazy	Clear	Clear	Clear	Clear	Clear	Clear
Temperature	°C	20.0	19.0	19.6	22.0	20.5	20.5	20.5	19.5	19.5	18.8
pH	-	6.73	7.19	6.53	6.70	6.73	6.77	6.87	6.82	6.53	7.76
Turbidity	NTU	1.5	6.9	1.0	90.0	1.2	4.5	1.0	0.9	1.4	0.8
Conductivity	µS/cm	1004	698	597	991	715	574	1448	634	431	315
T. Alkalinity as $CaC0_3$	mg/l	300	410	190	290	105	85	375	85	130	100
T. Acidity as $CaC0_3$	mg/l	95	87.5	105	75	70	15	100	55	75	5
T. Hardness as $CaC0_3$	mg/l	192	152	164	336	144	172	548	168	160	136
Ca Hardness as $CaC0_3$	mg/l	108	76	120	200	56	104	80	92	100	100
Mg Hardness as $CaC0_3$	mg/l	84	76	44	136	88	68	468	76	60	36
Calcium	mg/l	43.29	30.46	48.10	80.16	22.44	41.68	32.06	36.87	40.08	40.08
Magnesium	mg/l	20.47	18.53	10.71	33.14	21.46	16.57	114.8	18.53	14.62	8.76
Total Iron	mg/l	0.03	3.00	0.05	4.03	0.01	0.69	0.08	0.05	0.21	0.03
Total Ammonia	mg/l	80.00	1.04	12.00	60.00	0.10	0.52	96.0	0.04	0.12	0.08
Chloride	mg/l	134.9	284.0	78.1	99.4	92.3	49.7	149.1	71.0	42.6	21.3
DO	mg/l	2.02	5.27	2.63	1.01	5.67	4.86	2.43	6.27	4.45	6.27
BOD	mg/l	0.40	0.40	0.41	0.42	1.02	0.41	0.40	0.41	0.61	0.41

Table 15 (b) : Physico-Chemical Analysis of Protected Well Water Samples.

Parameters	Units	Sample Code (Sources)									
		WC11	WC12	WC13	WC14	WC15	WC16	WC17	WC18	WC19	WC20
Appearance	-	Clear	Clear	Clear	Clear	Slightly Hazy	Clear	Clear	Clear	Clear	Clear
Temperature	°C	19.8	18.8	18.8	18.8	20.0	21.4	19.3	20.0	20.0	20.2
pH	-	7.21	6.43	6.64	6.56	7.70	6.17	7.00	7.50	7.12	6.67
Turbidity	NTU	6.2	2.2	0.8	0.9	12.0	4.6	1.4	3.3	1.6	0.6
Conductivity	µS/cm	868	526	610	476	505	576	851	353	750	623
T. Alkalinity as $CaC0_3$	mg/l	270	115	115	125	150	75	165	150	385	130
T. Acidity as $CaC0_3$	mg/l	40	85	55	85	5	85	45	15	57.5	65
T. Hardness as $CaC0_3$	mg/l	256	132	136	140	176	120	134	108	292	156
Ca Hardness as $CaC0_3$	mg/l	196	78	66	96	132	100	60	68	192	120
Mg Hardness as $CaC0_3$	mg/l	60	54	70	44	44	20	74	40	100	36
Calcium	mg/l	78.56	31.26	26.45	38.48	52.91	40.08	24.05	27.25	76.95	48.10
Magnesium	mg/l	14.60	13.16	17.07	10.72	10.71	4.86	18.04	9.75	24.37	8.76
Total Iron	mg/l	0.11	0.03	0.11	0.21	0.21	0.03	0.16	0.03	0.08	0.08
Total Ammonia	mg/l	5.60	0.04	0.04	0.80	3.60	1.20	5.60	0.06	0.64	0.16
Chloride	mg/l	92.30	49.7	56.8	42.6	49.7	63.9	78.10	35.50	48.28	35.50
DO	mg/l	5.47	3.24	4.86	3.03	4.45	4.66	3.03	4.26	1.82	3.24
BOD	mg/l	1.61	0.40	0.41	0.60	0.80	0.61	0.80	0.21	1.01	0.20

Fig. 12: Graph Showing Iron and DO Values of Protected Well Water Samples

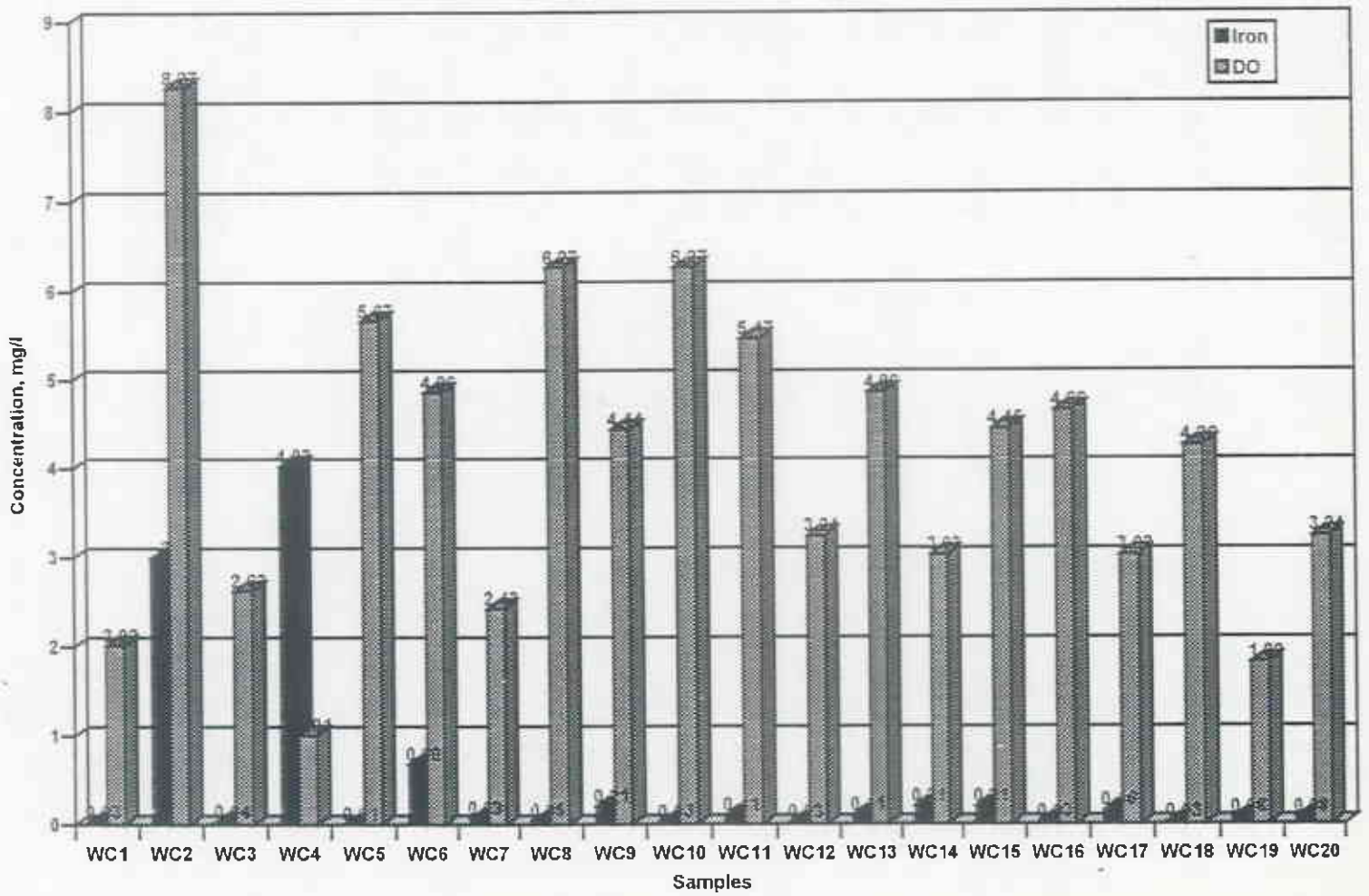


Fig. 13: Graph Showing Ammonia Values of Protected Well Water Samples

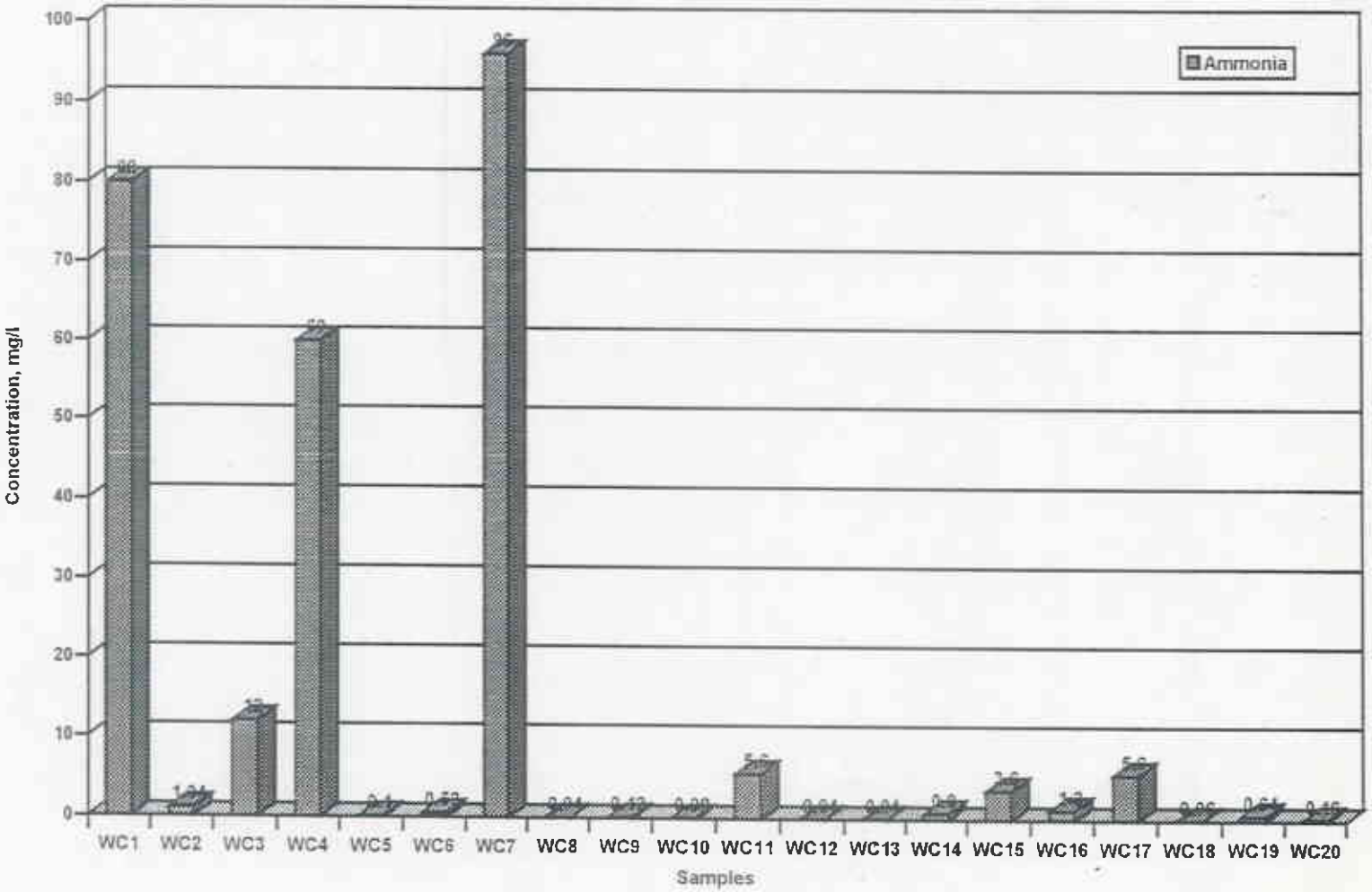


Table 16 (a) : Physico-Chemical Analysis of Unprotected Well Water Samples.

Parameters	Units	Sample Code (Sources)													
		WO1	WO2	WO3	WO4	WO5	WO6	WO7	WO8	WO9	WO10	WO11	WO12	WO13	WO14
Appearance	-	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Temperature	°C	18.2	19.0	17.5	19.3	20.0	20.8	18.5	19.8	18.0	20.4	19.9	19.5	19.0	18.0
pH	-	6.70	6.97	7.18	6.73	7.04	6.63	6.82	6.85	7.20	6.66	7.12	6.72	7.01	6.93
Turbidity	NTU	3.4	1.5	1.0	1.2	2.5	0.8	2.8	1.1	0.9	2.2	1.3	2.7	0.9	1.0
Conductivity	µS/cm	618	1004	1726	524	1139	557	504	703	1305	592	616	616	660	919
T. Alkalinity as $CaCO_3$	mg/l	190	260	230	175	260	120	95	105	190	115	120	95	145	100
T. Acidity as $CaCO_3$	mg/l	55.0	25.0	45.0	70.0	50.0	65.0	20.0	35.0	35.0	60.0	20.0	60.0	35.0	30.0
T. Hardness as $CaCO_3$	mg/l	204	204	250	168	112	464	130	104	222	328	136	156	108	198
Ca hardness as $CaCO_3$	mg/l	24	110	106	100	56	64	78	40	100	52	80	88	38	84
Mg Hardness as $CaCO_3$	mg/l	180	94	144	68	56	400	52	64	122	276	56	68	70	114
Calcium	mg/l	9.62	44.09	42.48	40.08	22.44	25.65	31.26	16.03	40.08	20.84	32.06	35.27	15.23	33.66
Magnesium	mg/l	43.92	22.91	35.11	16.57	13.66	97.59	52.18	15.61	29.75	67.33	13.65	16.57	17.07	27.80
Total Iron	mg/l	0.05	0.08	0.05	0.05	0.03	0.01	0.01	0.05	0.03	0.08	0.05	0.08	0.05	0.03
Total Ammonia	mg/l	0.64	96.0	1.60	4.00	2.24	0.04	0.06	0.04	1.60	0.04	0.02	0.06	0.08	0.06
Chloride	mg/l	71.00	134.9	213.0	56.80	149.1	56.80	49.70	92.30	177.6	63.90	63.90	71.00	71.00	113.60
DO	mg/l	5.27	4.05	4.66	4.25	4.66	5.28	5.68	7.09	4.86	6.43	6.48	6.27	2.63	7.09
BOD	mg/l	0.81	1.62	0.61	1.21	1.02	0.61	0.62	0.60	0.60	0.16	0.61	1.00	1.42	0.41

Table 16 (b) : Physico-Chemical Analysis of Unprotected Well Water Samples.

Parameters	Units	Sample Code (Sources)													
		WO15	WO16	WO17	WO18	WO19	WO20	WO21	WO22	WO23	WO24	WO25	WO26	WO27	WO28
Appearance	-	Clear	Clear	Clear	Clear	Clear	Clear	Slightly Hazy	Clear	Clear	Clear	Clear	Clear	Slightly Hazy	Clear
Temperature	°C	19.1	19.5	19.0	19.7	21.0	20.5	20.2	20.8	19.0	20.3	21.4	19.0	20.2	20.4
pH	-	6.44	6. 80	6.89	6.16	6.63	6.97	7.02	7.00	6.53	7.00	6.69	6.50	6.63	6.71
Turbidity	NTU	1.0	0.7	2.0	1.7	1.7	3.1	16.0	1.8	3.0	1.2	0.9	2.2	12.0	2.0
Conductivity	µS/cm	411	1438	698	1065	596	430	363	248	566	992	886	669	950	1012
T. Alkalinity as $CaC0_3$	mg/l	110	220	325	425	130	160	135	130	75	130	250	75	295	225
T. Acidity as $CaC0_3$	mg/l	85.0	70.0	80.0	65.0	50.0	35.0	30.0	30.0	40.0	30.0	70.0	50.0	115.0	90.0
T. Hardness as $CaC0_3$	mg/l	144	204	236	356	148	128	108	116	140	132	220	152	232	188
Ca Hardness as $CaC0_3$	mg/l	88	120	160	212	100	76	80	88	80	64	168	128	156	136
Mg Hardness as $CaC0_3$	mg/l	56	84	76	144	48	52	28	28	60	68	52	24	76	52
Calcium	mg/l	35.27	48.10	64.13	84.97	40.08	30.46	32.06	35.27	32.06	25.65	67.33	51.30	62.52	54.51
Magnesium	mg/l	13.65	20.47	18.51	35.09	11.69	12.67	6.82	6.81	14.63	16.58	12.66	5.83	18.52	12.66
Total Iron	mg/l	0.11	0.05	0.16	0.01	0.64	0.03	0.03	0.08	0.48	0.53	0.01	0.21	0.05	0.01
Total Ammonia	mg/l	0.16	1.40	96.00	50.00	0.06	0.04	2.40	1.20	42.50	0.20	56.00	0.72	13.00	96.0
Chloride	mg/l	42.60	156.2	63.90	106.5	56.80	28.40	28.40	14.20	42.60	113.6	106.5	71.00	113.6	106.5
DO	mg/l	3.04	3.64	4.05	1.62	6.47	3.24	2.64	5.06	5.47	8.90	2.63	3.44	3.41	2 .63
BOD	mg/l	0.40	0.61	0.82	0.41	0.39	0.40	0.62	0.40	1.61	1.81	0.41	0.41	1.40	0.41

Fig. 14: Graph Showing Iron and DO Values of Unprotected Well Water Samples

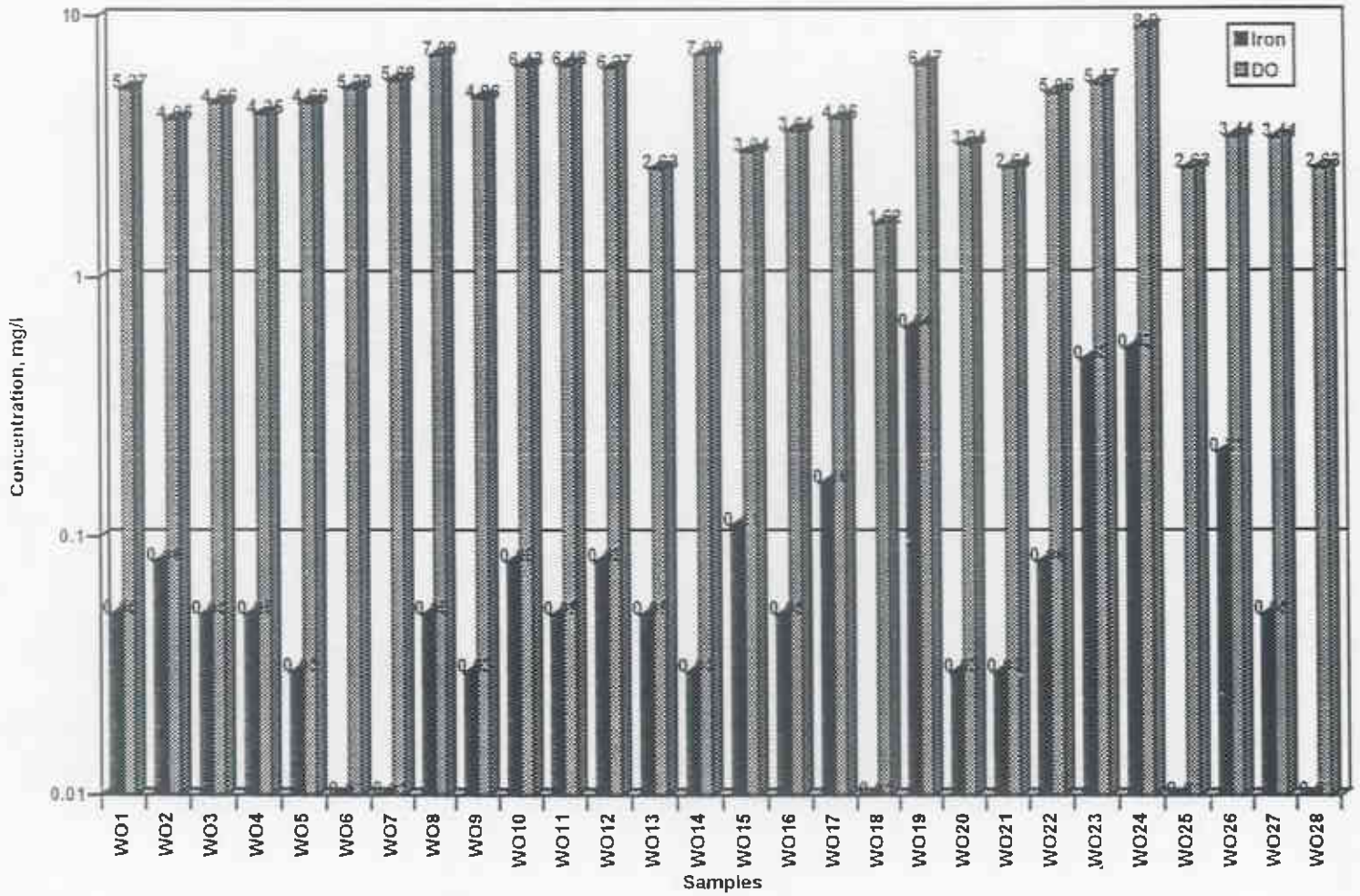
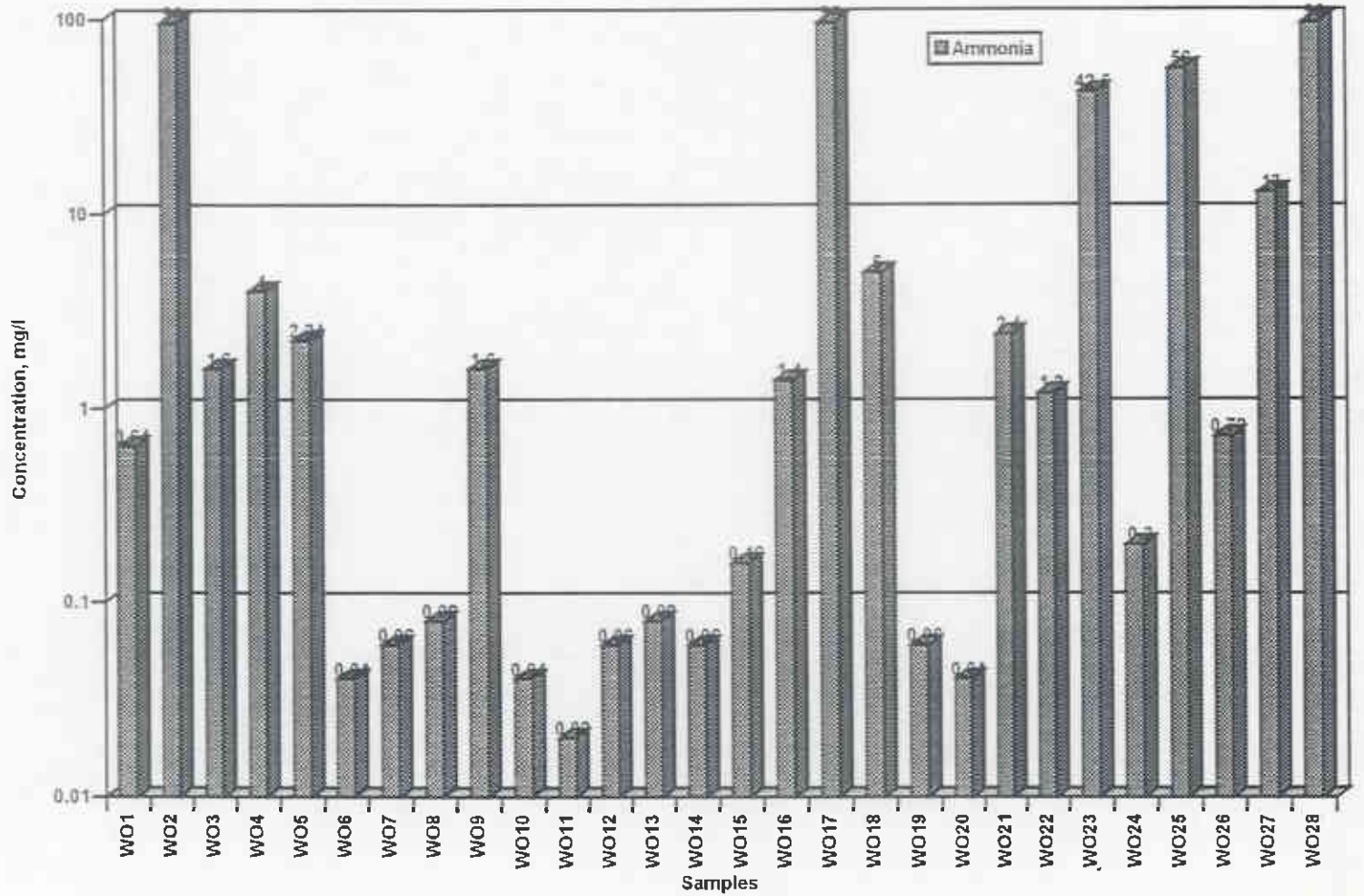


Fig. 15: Graph Showing Ammonia Values of Unprotected Well Water Samples.



4.4 Frequency of Antibiotic and Multiple-Antibiotic Resistance Among Enterobacteria Isolates

A total of 120 enterobacteria isolates were assayed for resistance to ten antibiotics. Antibiotic sensitivity patterns of isolated enterobacteria were shown in Appendix -II. Resistance was most commonly directed toward nitrofurantoin, ampicillin and tetracycline with nearly 30% or more of the strains expressing resistance to at least one of these antibiotics. Nearly 20% and more than 10% of the isolates were resistant to chloramphenicol and co-trimoxazole respectively. Less than 10% were resistant to nalidixic acid and ceftazidime, nearly 1% to ciprofloxacin and none to amikacin and gentamycin. The maximum resistance was observed for nitrofurantoin (68.3%) followed by ampicillin (44.2%) and tetracycline (28.3%). Percentage resistance pattern to each individual antibiotic among 120 enterobacteria isolates was given in Table 17. Isolates were characterized as AR and MAR frequencies (Table 18). Out of total 82.5% isolates were resistant to at least one antibiotic. MAR was expressed by 54.2% of all isolates, and ranged as high as 100% for some species. Of the MAR organisms isolated, 2.5% were resistant to five or more antibiotics. The organism (SS32) exhibited resistance to seven antibiotics (Plate 11) which is the only case encountered to resist maximum number of antibiotics tested in the study.

4.5 Study of Oligodynamic Action

Five types of metals (Silver, copper, brass, aluminium and steel) were tested for their inhibitory effect against *E. coli*, *Salmonella sp.*, *Shigella sp.* and *Klebsiella sp.* isolates. Zone of inhibition (ZOI) was observed for silver, copper and brass after 48 hours of incubation period. Size of zone of inhibition for silver was maximum followed by copper and brass, sequentially. But, zone of inhibition was not observed for aluminium and steel (Plate 13). In comparison, silver and copper were found very effective, brass less effective, while aluminium and steel ineffective for their inhibitory effect aganist tested organisms. It is noteworthy that size of zone of inhibition increased with the increase of incubation period. The inhibitory effect pattern observed was almost same for all the tested organisms in this study (Fig. 18).

Table 19 : Inhibitory Effect Patterns of Various Metals Against Bacteria

Metals	Average ZOI (mm)	Organisms Tested
Silver (Ag)	45	*E. coli*,
Copper (Cu)	34	*Salmonella sp.*,
Brass (Brs)	28	*Shigella sp.*, and
Aluminium (Al) & Steel (Stl)	0	*Klebsiella sp.*

Table 17 : Antibiotic Resistance Among 120 Groundwater Isolates

Antibiotic	% Resistant
Nitrofurantoin (300µg)	68.3
Ampicillin (10µg)	44.2
Tetracycline (30µg)	28.3
Chloramphenicol (30µg)	19.2
Co-trimoxazole (25 µg)	11.7
Nalidixic Acid (30µg)	5.0
Ceftazidime (30µg)	4.2
Amikacin (30µg)	-
Gentamycin (10µg)	-
Ciprofloxacin (5µg)	0.8

Table 18 : Frequency of Antibiotic and Multiple-Antibiotic Resistance among Enterobacteria Isolates

Organisms	AR[a]	MAR[b]	5/more[c]
E. coli	15 (71.4)	5 (23.8)	-
E. coli, inactive	2 (100.0)	2 (100.0)	1 (50.0)
Escherichia blattae	-	-	-
Enterobacter aerogenes	19 (90.5)	13 (61.9)	-
Enterobacter agglomerans	7(50.0)	5 (35.7)	-
Enterobacter sakazakii	1(100.0)	-	-
Citrobacter sp.	5 (100.0)	5 (100.0)	-
Citrobacter freundii	10(100.0)	9 (90.0)	1(10.0)
Klebsiella pneumoniae	1(100.0)	1(100.0)	1(100.0)
Klebsiella oxytoca	1(100.0)	1(100.0)	-
Salmonella sp.	7(100.0)	2(28.6)	-
Salmonella paratyphi A	4 (66.7)	2(33.3)	-
Shigella dysenteriae	5 (83.3)	2(33.3)	-
Morganella morganeii	1(100.0)	1(100.0)	-
Providencia alcalifaciens	1(100.0)	1(100.0)	-
Serratia marcescens	3(100.0)	3(100.0)	-
Serratia fonticola	1(100.0)	1(100.0)	-
Hafnia alvei	9(100.0)	7(77.8)	-
Yersinia enterocolitica	4(100.0)	4(100.0)	-
Yersinia frederiksenii	1(50.0)	1 (50.0)	-
Yersinia kristensenii	1(100.0)	-	-
Yersinia intermedia	1(100.0)	-	-
Total	98(81.7)	65(54.2)	3(2.5)

[a]Resistant to at least one antibiotic.
[b]Resistant to at least two antibiotics.
[c]Resistant to at least five or more antibiotics.
Figures in parentheses are percent recovery of resistant isolates within genus/species.

Fig. 16: Percentage Sensitivity Pattern to Each Individual Antibiotic Among 120 Enterobacteria Isolates.

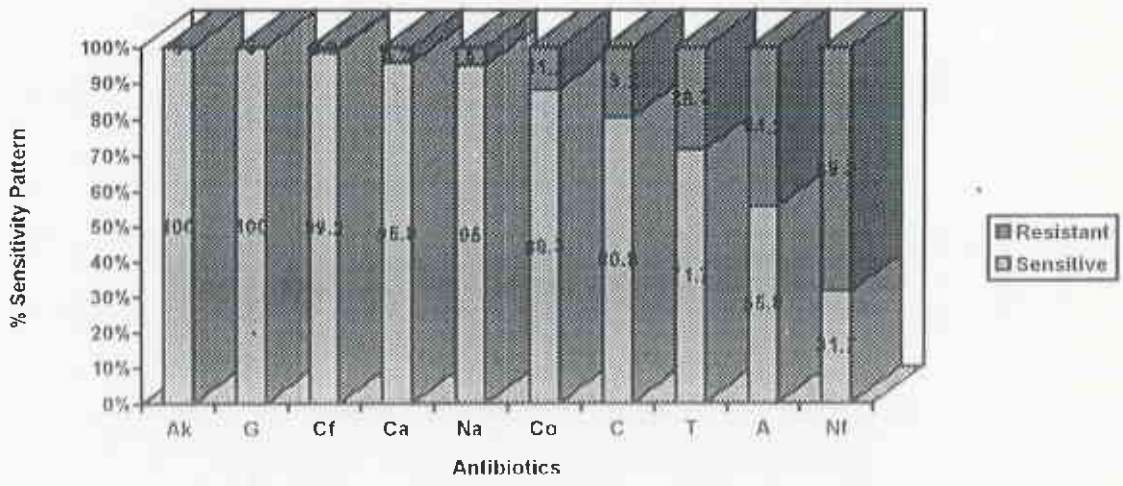


Fig. 17: Antibiotic Sensitivity and Resistance of Enterobacteria Isolates in Percentage

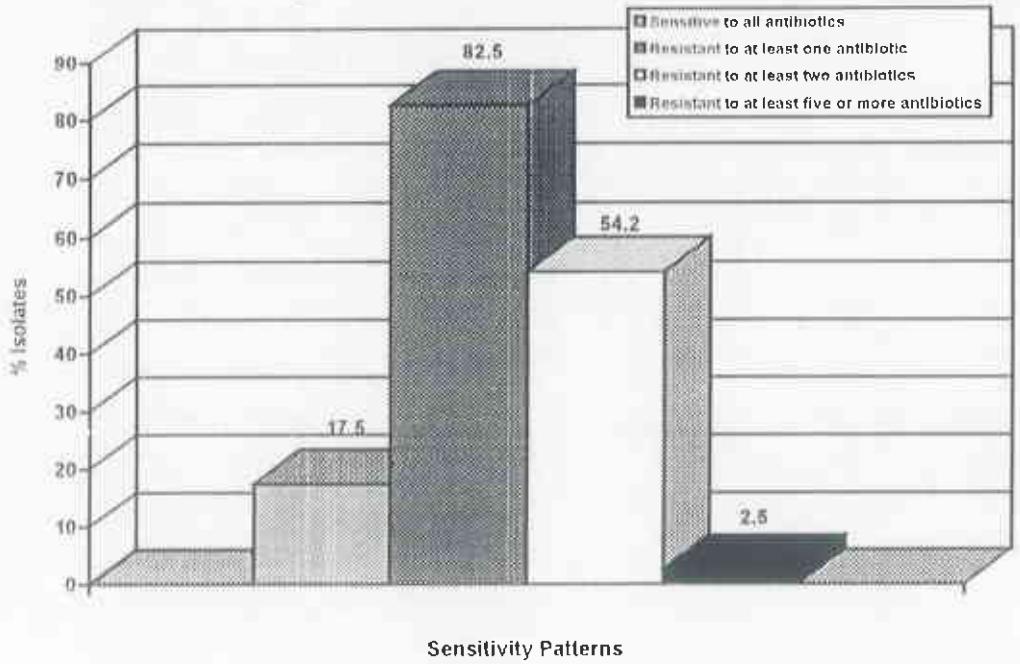
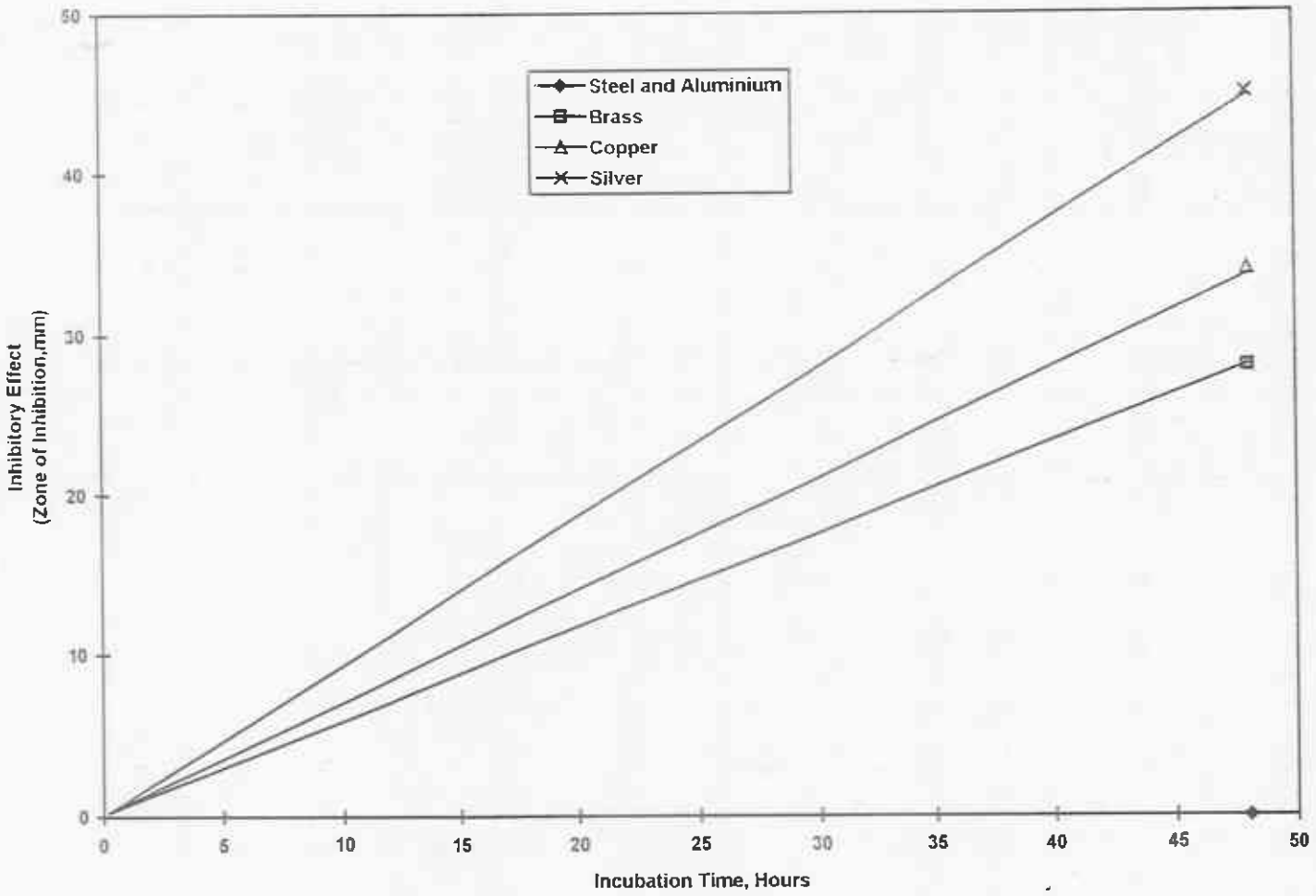


Fig. 18: Patterns of Inhibitory Effect of Various Metals against Some Enterobacteria Isolates



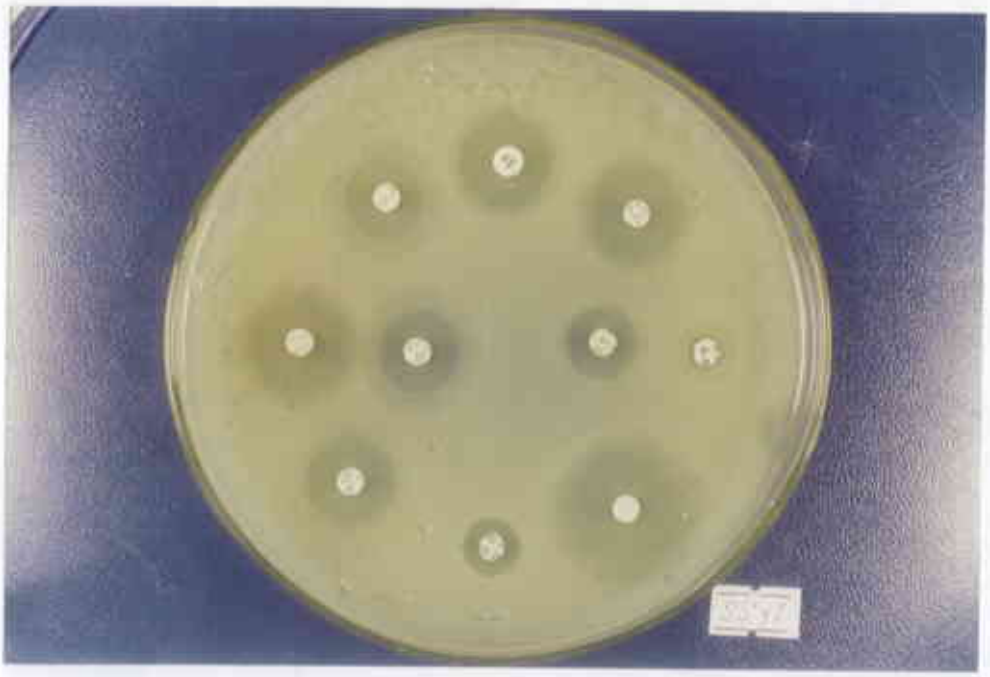


Plate 8 : Antibiotic Resistance Pattern Shown by *E. coli* ($SS3_1$)

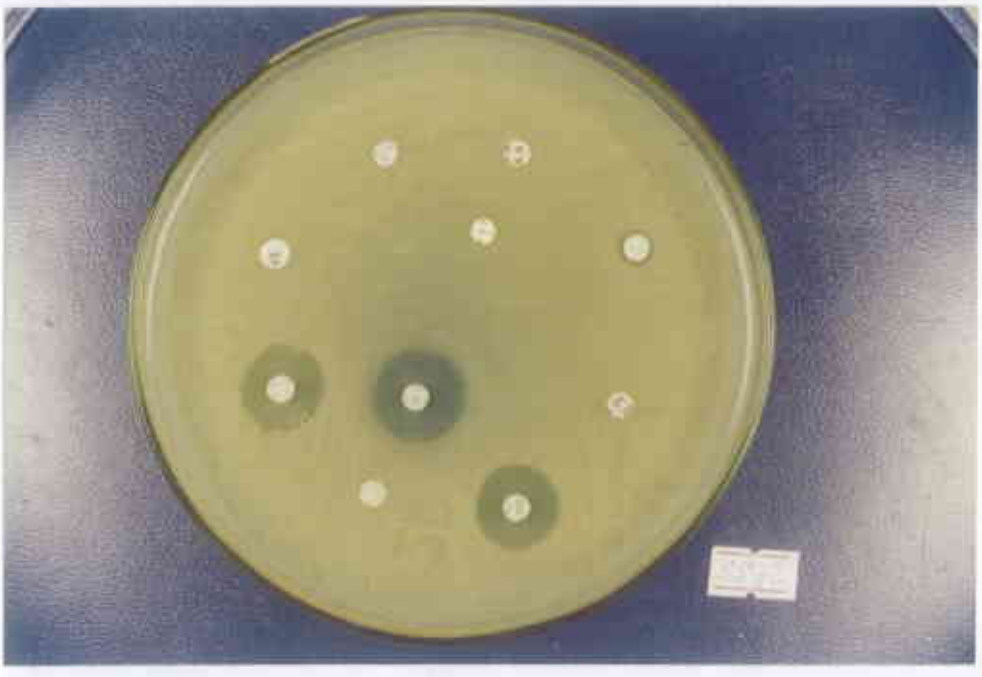


Plate 9 : Antibiotic Resistance Pattern Shown by *Klebsiella sp.* ($SS3_2$)

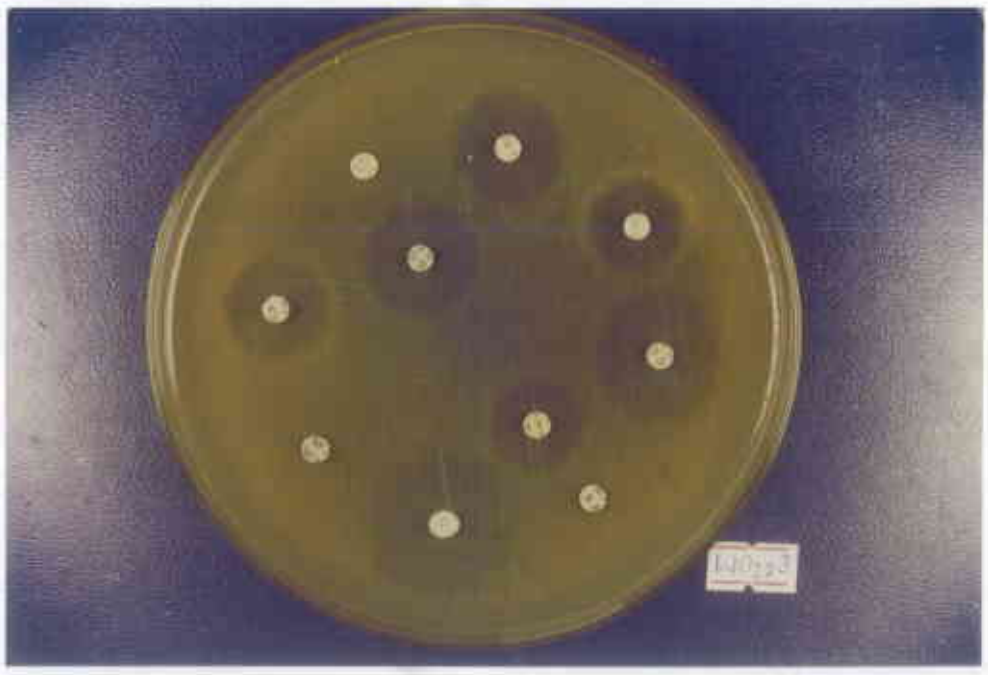


Plate 10 : Antibiotic Resistance Pattern Shown by *Shigella sp.* ($WO17_3$)

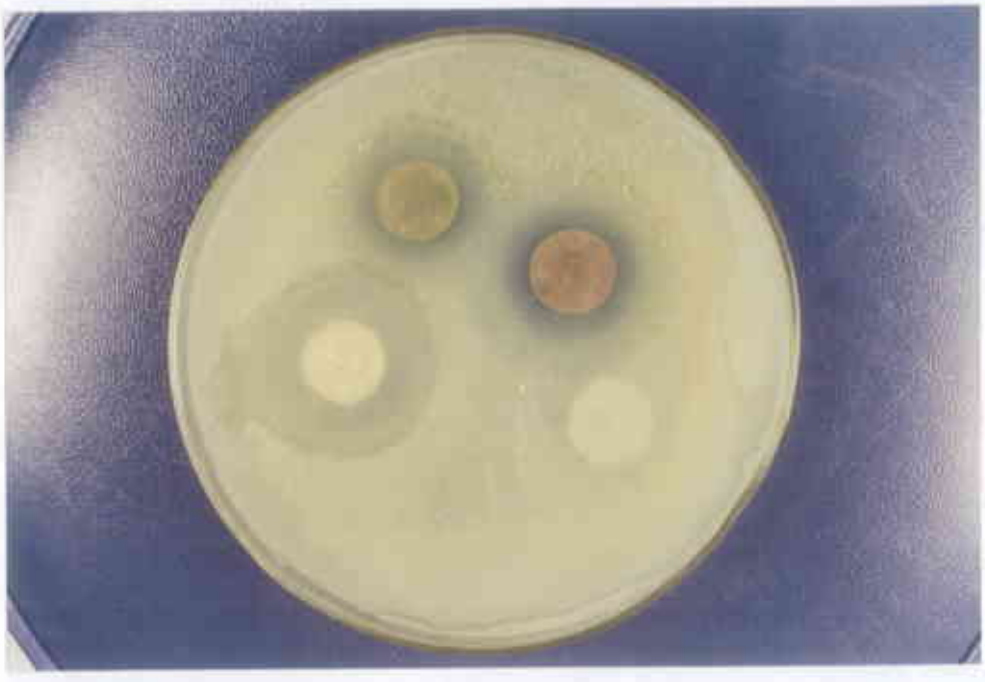


Plate 11 : Inhibitory Effect Pattern of Various Metals Against Bacteria (Some Enterobacteria Isolates)

Results of Survey Work

Sources of Water : During this survey an acute water shortage problem was clearly observed in urban area of Patan. The traditional groundwater sources were noted as significant alternative for the people to meet their water needs.

Table 20 : Water Sources and Sanitary Condition

Sources	Dug Wells		Shallow Wells				
	WC	WO	SWC	SWO	SP	SS	Total
	20	28	1	2	5	14	70
Sanitary Condition	G -18.6% P -10.0%	G- 20.0% P - 20.0%	- P -1.4%	- P-2.8%	G -2.9% P-4.3%	G -7.1% P- 12.9%	G-48.6% P-51.4%

WC = Protected Well WO = Unprotected Well SWC = Protected Shallow Well
SWO = Unprotected Shallow Well SP = Shallow Pump SS = Stone Spout
G = Good P = Poor

Table 20 shows that a total of 70 water sources including 48 dug wells(20 protected wells and 28 unprotected wells), 3 shallow wells (1 protected and 2 unprotected), 5 shallow pumps and 14 stone spouts were analyzed in the present study. Out of which 48.6% were good from sanitary points of view and rest (51.4%) were in poor condition. All the aquifers were observed unhygienic in terms of sanitary condition.

Table 21 : Percentage of Sources in Use and not in Use

Sources	Dug Well		Shallow Wells				
	WC	WO	SWC	SWO	SP	SS	Total
Drinking	25.7%	32.9%	1.4%	-	7.1%	20.0%	87.1%
Not Drinking	2.9%	7.1%	-	2.9%	-	-	12.9%

During this study it was found that 87.1% of the total sources were used for drinking and others (12.9%) not for drinking. Groundwater sources were found to be extensively used by the local residents for all their needs including drinking.

Plate 12 : A Clear Evidence of Dependence of People on Dug Wells for Water

Plate 13 : A Clear Evidence of Dependence of People on Stone Spouts for Water

Table 22 : Drinking Water Storage Vessels in Percentage

Brass	Copper	Aluminium	Plastic	Steel	Clay
238 (39.0)	8 (1.3)	192 (31.5)	172 (28.2)	-	-

Figures in the parentheses are percentage of water storage vessels.

Table 22 shows that people used different types of vessels for drinking water storage. The use of brass vessels was found maximum (39.0%), followed by aluminium (31.5%), plastic (28.2%) and copper (1.3%), sequentially. During this survey none was using steel and clay vessels.

Table 23 : Water Treatment in Percentage

Treatment	Bleaching Powder	Chlorination	Potash	Boiling	Using Filter
At Source (Dug Wells and Shallow Wells only)	34 (48.6) but irregularly	-	-	-	-
At Home	-	-	-	5 (0.8)	35 (5.7)

Put cover on water vessels at home - 518 (84.9%).
Don't put cover on water vessels at home - 92 (15.1%).

From this survey it was found that people used to treat 48.6 % of the total sources with bleaching powder but they do it irregularly. People donot treat the water at home before consuming except very few. However, majority of them 84.9% put cover on the drinking water vessels at home.

Table 24 : Percentage of Families Suffering with Diseases

Common Cold	Diarrhoea	Dysentery	Typhoid	Ascariasis	Pneumonia	Jaundice	Frequency
High Rate	60.0%	3.4%	3.0%	16.0%	-	-	*

* 6-7 times per year among family members

The occurrence of waterborne diseases among water consumers was found. The people informed that they use to suffer from common cold (flu) most often. Diarrhoea rate was high (60.0%) followed by other disorders as shown in Table 24.

Table 25 : Water Source, Quantity and Quality

Households with Tap at Home	Households Dependent on Groundwater Sources	Quality of Groundwater to the users
315 (45.0%) but no adequate supply and water sometimes dirty	Most often - 165 (23.5%) Only when tap turns dry - 95 (13.6%) Throughout the year - 440 (62.9%)	Satisfactory - 55% Unsatisfactory - 45%, offensive and visible organisms sometimes

From this survey it was found that water supply from centralized system in the city was inadequate even to meet the minimum demand of the people. This is clear from the plate 12 and 13. About 63.0% of the people depend on groundwater sources throughout the year. Only 55.0% of the people interviewed were satisfactory to the water quality while rest 45.0% were unsatisfactory.

CHAPTER - FIVE

DISCUSSION

This study was undertaken to assess the existing status of physical, chemical and bacteriological quality of various groundwater sources in Patan city, an urban area south of Kathmandu. In addition, the prevalence of antibiotic resistant enterobacteria, antibiotic resistance pattern and inhibitory effect of some heavy metals were also studied. A total of 70 water samples collected from dug wells, shallow wells, shallow pumps and stone spouts were analyzed.

In Patan, the urban population is still dependent on traditional groundwater sources (stone spouts, wells) as the growing population uses more water than the reticulated system can supply. The piped water supply, although preferred by most people, is intermittent and flow rates are slow, especially in the dry season. The piped system has never been sufficient to meet even the minimum demand of the people, besides unacceptable quality of water. Thus, many people in Patan have no choice but to use well water or stone spouts for all their needs including drinking since these are easily available sources that can deliver water round the clock. There is an increased dependency on spouts and wells compared to before especially in the dry season when taps turn dry altogether in some places. There are some parts of the area where spout or well water is exclusively used all year round. A regular monitoring and surveillance of local groundwater sources are, therefore, needed to check up the degree of pollution, its causes and necessary measures to be taken to ensure public health safety. But, very few studies have been carried out in spite of the increased dependency on them. With this view the present work was undertaken to reveal the existing quality of the water supplied by groundwater sources in Patan city. No studies, to the authors knowledge, have been conducted on resistance of bacteria, recovered from groundwater sources, to antibiotics and inhibitory effect of heavy metals in Nepal as whole. I hope the present work will add one milestone to the relevant study of its kind.

Clean, protected and safe water is a basic need for a healthy living. Its significance in ensuring public health has been internationally recognized and stressed by drinking water and sanitation decade (1981-1990) launched by the countries members of the United Nations. But, water pollution, today, has bacome a global problem and the greatest impact of water pollution on public health comes through drinking water. Pollution of water results from the wide range

of human activities and intrusion of sewage, or of pollutants into drinking water supplies from point and non-point sources. Thus, water, a vital resource to humans, can be extremely dangerous when it becomes the vehicle of transmission of disease and causes outbreaks of such disease which may be widely disseminated. Epidemic waterborne diseases cause emotional and economic losses in communities affected.

5.1 Bacteriological Quality

The water samples analyzed from the public taps and treatment plants of the distribution system at various parts of the country, including Kathmandu has revealed that the water supply system in Nepal lacks proper disinfection. Almost all of the studies carried out to date suggest that the water quality is deteriorating with time in all types of sources. All studies (Sharma, 1978; Sharma 1986; Adhikari *et al.*, 1986; CEDA, 1989; ENPHO/DISVI, 1992; ENPHO/DISVI, 1995; Sharma, 1993) indicate that the public water supply is far from satisfactory in almost all localities in terms of bacterial contamination.

Sharma (1978) had found only 50% samples contaminated with faecal material in Kathmandu. In a follow up study, Sharma (1986) found that the level of coliform contamination had significantly increased (85%) in between nine years. Sharma (1993) found 33.3 to 16.7% and 70 to 100% samples contaminated with coliforms in rural and urban areas of Nepal, including Kathmandu respectively. Similarly, Adhikari *et al.* (1986) found 88% of the samples unsatisfactory. Likewise, CEDA (1989) recorded almost all the water samples tested to be contaminated with faecal material. Joshi (1987) found coliform bacteria in water sources of Chaubas and Syabru villages near to the Kathmandu, Nepal. Pradhananga *et al.* (1993) found average coliform count ranging from 8 col/100ml to 88 col/100ml in six stone spouts around Pashupati area in 1992. Joshi *et al.* (1992) recorded 8 out of 11 spouts in Patan to be contaminated with coliform organisms. Similarly, 29 well samples tested from Patan city in 1991 exhibited high coliform count (Lewis, 1995). Ghimire (1996) also found majority of the samples from spouts and wells in Patan faecally contaminated. Thapa (1997) recovered high coliform densities in all samples from Baluwa VDC, near to the Kathmandu city.

ENPHO/DISVI (1992) found 18% samples from treatment plants and 50% from public taps to be contaminated with faecal coliforms. Similarly, ENPHO/DISVI (1995) recovered 49% samples contaminated in Patan and contamination level in Kathmandu city Water Supply reached maximum (about 88%) during the month of May in 1995. ENPHO/DISVI (1990) found 81% of

the spouts in Kathmandu to be highly contaminated with faecal material. Studies conducted by ENPHO/DISVI (1990) on drinking water quality in Terai tubewell project in seven rural areas of the Eastern Development Region of Nepal and ENPHO/DISVI (1991) in Pokhara and Siraha district showed majority of the samples to be faecally contaminated.

The results of this study also showed most of the sources to be faecally contaminated. Out of total 85.6% of the samples showed the presence of total coliforms, while 68.6% exhibited faecal coliforms. All shallow pumps, two stone spouts, one protected and two unprotected dug wells were found not contaminated with faecal material at the time of study. The densities of total and faecal coliforms ranged from 160 to 880 CFU/100ml and 40 to 800 CFU/100ml, respectively for shallow well water samples, and 25 to 7840 CFU/100ml and 21 to 3660 CFU/100ml, 2 to 290 CFU/100ml and 1 to 280 CFU/100ml, and 10 to 960 CFU/100ml and 2 to 520 CFU/100ml for stone spouts, protected and unprotected wells respectively. In overall, 68.6% of the total water samples were found to exceed the WHO permissible level containing total coliforms (>10 CFU/100ml) and faecal coliforms. The results of this study in regard to bacteriological quality of the drinking water are similar with those of Joshi (1987), ENPHO/DISVI (1990). Pradhananga *et al.* (1993), Sharma (1993), Joshi *et al.* (1992), Lewis (1995), Ghimire (1996) and Thapa (1997).

Studies on drinking water quality in other countries conducted by Antai (1987) in Port Harcourt (Nigeria), Yulug and Tug (1988) in Ankara (Turkey), Ibiebele and Sokari (1989) in Port Harcourt (Nigeria), Hosny *et al.* (1990) in Cairo (Egypt), Combarro *et al.* (1988) in Galicia (Spain), Wadud *et al.* (1992) in Pakistan, Khan and Khan (1992) in Mardan Division (Pakistan) and Mahasneh (1992) in Jordan showed significant levels of bacterial contamination. The presence of indicator organisms beyond the standard values were also detected by Eldin *et al.* (1993) in Saudi Arabia, Somasundaram *et al.* (1993) in Madras (India), Pathak and Gopal (1994) in India, and Rai and Sharma (1995) in North-West Uttar Pradesh, India.

5.2 Recovery of Enteric Bacteria

The present study isolated 120 enterobacteria from 49 out of 70 groundwater sources. The identified organisms include different 11 species of enteric bacteria. Some of the organisms recovered and identified are highly pathogenic. Recovery of *Enterobacter sp.* (30.0%) was maximum followed by *E. coli* (20.8%), *Citrobacter sp.* (12.5%), *Salmonella sp.* (10.8%), sequentially, and others. Maximum enterobacteria isolates (45.9%) were recovered from unprotected wells

indicating open wells are more liable to pollution than protected ones. During this study, *Enterobacter sp.* was isolated from 32 samples, and *E. coli, Citrobacter sp., Salmonella sp., Shigella sp.* and *Klebsiella sp.* from 24,15,12, 6 and 2 samples, respectively. Similarly, *Hafnia alvei, Yersinia sp. , Serratia sp., Morganella morganii* and *Providencia alcalifaciens* was isolated from 9,8,4,1 and 1 samples, respectively. Sharma (1993) also isolated enteric pathogens viz. *E. coli, Proteus sp., Salmonella sp.* and *Klebsiella sp.* from water samples. Thapa (1997) also isolated *E. coli, Salmonella sp.* and *Klebsiella sp.* Similarly, Antai (1987), Yulug and Tug (1988), Ibiebele and Sokari (1989), wadud *et al.* (1992), Pathak and Gopal (1994), and Bissonnette *et al.* (1995) detected different species of enteric bacteria including pathogenic ones from various groundwater sources in Port Harcourt (Nigeria), Ankar (Turkey), Port Harcourt (Nigeria), Brasil, Peshawar (Pakistan), India and northern West Virginia (USA), respectively.

All of the above studies clearly indicated that most of the natural water resources are faecally contaminated. The results of this study revealed that nearly 70% of the groundwater sources in Patan were unacceptable having high coliform bacteria (>10CFU/100ml) during this study (Table 10). Pathogenic bacteria of medical significance were also detected from widely used local groundwater sources (Table 11). Many people in Patan have no choice for drinking water but to use contaminated dug wells, spouts, shallow wells or shallow pumps for all domestic purposes including drinking. There is high risk of outbreak of water borne disease at any time. Thus, serious attention and measures should be urgently taken to ensure safe water.

5.3 Physico-Chemical Approach

The previous studies have found most of the physico-chemical parameters of drinking water to lie within the WHO guideline value, and some parameters either above or below the permissible level. DISVI (1990), ENPHO/DISVI (1990), and ENPHO/DISVI (1991) found most of the parameters within the prescribed level, except slight fluctuation of the values for some parameters in seven rural areas of Ilam in Eastern Nepal, of 21 spouts in Kathmandu city and in Terai Tubewell Project in seven rural areas of the Eastern Development Region of Nepal, and in Siraha district respectively. Similarly, Pradhanaga *et al.* (1993), Ghimire (1996) and Thapa (1997) found the values within safety limit set by WHO (1984) for most of the parameters. The concentrations of iron, ammonia and total hardness were found above the recommended level in some cases. It is noteworthy that 71% of the spouts in Kathmandu were recorded to exceed the safety limit for ammonia.

The results of the present study are also similar to the above studies. From tables 12 to 16, it was found that almost all (91.4%) sources contain water of clear appearance. The temperature of the water samples ranged from 17.5°C to 25.0°C. A rise in temperature of the water leads to the speeding up of the chemical reactions in water, reduces the solubility of gases and amplifies the tastes and odours. pH indicates acidic or basic nature of water. Waters from most of the sources are almost neutral as indicated by the pH values. During this study, 88.6% of the samples were recorded within permissible limit of pH value, while rest showed slightly low pH values.

Turbidity indicates clarity of water and is caused by living and non-living suspended matter and colour producing substances. The values for turbidity from most of the sources (87.1%) lie within the WHO standard (5NTU), while rest exceeded the acceptable level. In this study, 94.3% samples showed the values for conductivity within maximum allowable limit proposed by NWSC.

The values for total alkalinity and acidity were found in between 75 mg/l to 425 mg/l and 5mg/l to 175 mg/l, respectively. Alkalinity in natural waters is due to free hydroxyl ions and hydrolysis of salts formed by weak acids and strong bases. Determination of alkalinity is significant as it produces undesirable taste and soapy feel, while acidity causes corrosion and influences the chemical and biochemical reactions.

Hardness of water reflects the composite measure of the polyvalent cation concentration in water. Principal cations imparting hardness are calcium and magnesium. High hardness causes scale deposition and scum formation, while low hardness causes corrosion. In this study, 95.7% of the samples were found within the acceptable limit proposed by NWSC, and WHO standard as well. Similarly, calcium hardness and magnesium hardness ranged from 24mg/l to 212 mg/l and 12 mg/l to 468 mg/l, respectively.

The principal sources of calcium and magnesium in the natural waters are various kinds of rocks. Sewage and industrial wastes are also important sources of calcium and magnesium. The concentration of magnesium in water remains generally lower than the calcium. High concentrations of calcium and magnesium contribute to the hardness of the water. High concentrations of magnesium combined with sulphate causes gastrointestinal irritation. During this study,

92.9% of samples showed fairly low values for calcium, and magnesium content was also found below the recommended value proposed by NWSC for 87.1% samples.

Iron in drinking water is more significant in its aesthetic and taste consideration rather than in health aspects. High concentration of iron in water stains laundry, sanitary wire and gives an undesirable taste, and develops turbidity of water as well. Out of total 14.3% of the sources showed iron values within WHO standard, 12.9% exceeded the acceptable limit and 72.8% with fairly low values. However, if the guideline value proposed by NWSC is followed, 22.9% of the sources were found to be acceptable and 4.3% unacceptable, while 72.8% with the values below the prescribed level.

Sewage has large quantities of nitrogenous matter, thus its disposal tends to increase the ammonia content of the water. Occurrence of ammonia in excess is the indication of organic pollution, and in groundwaters it is quite generally a result of natural degradation processes. Ammonia in higher concentration is toxic to man, fish and other biota. The total ammonia values in this study ranged from fairly low to extremely high exceeding the maximum permissible level. Among the water samples analyzed, 38.6% were found to exceed the limit indicating organic pollution of the sources.

The chloride content of almost all samples (98.6%) were found to lie within WHO standard, except for one which exceeded the recommended value of 250 mg/l.

Dissolved oxygen is one of the most important parameters in water quality assessment and reflects the physical and biological processes prevailing in the waters. DO present in water is important and necessary for aquatic life. Low DO concentration in water indicates heavy contamination of the sources with organic matter. Higher the DO value, better is the water quality. In this study, levels of DO were noted in between 1.01 mg/l and 8.90 mg/l.

Biological Oxygen Demand (BOD) is a measure of the amount of oxygen consumed by indigenous microbial population in water. The higher the value of oxygen consumed the higher is the organic matter content. The values for BOD were recorded in a range of 0.20 mg/l to 1.81 mg/l during this study.

5.4 Resistance of Bacteria to Antibiotics

Antibiotics are used to counteract bacterial infections, but the extensive use and misuse of antimicrobial drugs have favoured the emergence of resistant strains. In under - developed countries like Nepal, most of the people are uneducated and do not know about the causes that may lead to the development of resistant bacterial strains. The inappropriate or indiscriminate use of antimicrobials and their being taken at wrong dosages and for an insufficient length of time and repeated exposure of bacteria to antibiotics are some of the major causes responsible for the resistance development. The increasing ineffectiveness of drugs combined with the unavailability of alternative antimicrobials can contribute to the spread of major infectious diseases causing serious epidemics.

No studies have been conducted on resistance of bacteria, isolated from groundwater supplies, to antibiotics in Nepal before. But, many studies have been done in other countries. Chugh and Suheir (1983) studied drug resistance among *Salmonella sp.* prevalent in Kuwait. They found percent resistant to tetracycline, kanamycin, ampicillin, and chloramphenicol as 69,61,56 and 38, respectively. MAR was observed in 71% of isolates. Similarly, Antai (1987) studied antibiotic resistance of *E. coli* strains isolated from water samples in Port Harcourt (Nigeria), MAR was observed in the study. El-Zanfaly *et al.* (1987) and Hosny *et al.* (1988) examined underground water isolates in Cairo (Egypt) for their resistance to antibiotics MAR was observed, and most isolated strains were found resistant to ampicillin, tetracycline and chloramphenicol. Similarly, antibiotic resistance of coliforms isolated from different drinking water sources and from different places in India were carried out by Ramteke *et al.* (1991), Pandey and Musarrat (1993), and Pathak and Gopal (1994). MAR was observed, and resistance to ampicillin was found more prevalent among the coliform isolates. Bissonnette *et al.* (1995) examined 265 isolates from groundwater supplies in West Virginia (USA) for antibiotic resistance. MAR was observed, and percent resistant to ampicillin, nitrofurantoin, tetracycline, chloramphenicol and nalidixic acid were found as 69.4, 47.7, 32.3, 16.9 and 12.0 respectively, while below 10.0 for amikacin and gentamycin.

In this study, ten antibiotics were tested against 120 enterobacteria isolates. Table 17 shows that resistance was most commonly directed toward nitrofurantoin, ampicillin and tetracycline. All the isolates were sensitive to amikacin and gentamycin. 82.5% of all isolates were resistant to at least one antibiotic, while MAR was expressed by 54.2%. Of the MAR organisms, 2.5% were resistant to five or more antibiotics (Table 18). The results of this study are almost

similar with those of El-Zanfaly *et al.* (1987), Hosny *et al.* (1988), Ramteke *et al.* (1991), Pandey and Musarrat (1993), Pathak and Gopal (1994), and Bissonnette *et al.* (1995).

5.5 Oligodynamic Action

ENPHO/DISVI (1991) and Shahi *et al.* (1996) observed a significant decrease in bacterial count with time in water stored in the metallic pots, particularly in silver coated copper, copper and brass, while steel and aluminium were found less effective.

The results of this study found silver and copper to be very effective, comparatively brass less effective, while aluminium and steel ineffective for their inhibitory effect against tested organisms (Fig. 18). The observed zone of inhibition for silver, copper and brass might be due to the disinfecting ability of the metals. The germicidal action of metals is due to oligodynamic characteristic imparted by metals. The results of this study therefore suggest storing drinking water in metallic vessels (silver, copper and brass) purify the water. Thus, storing water in metallic pots is safe than improper chemical treatment since overdose or underdose use of chemicals in water treatment can have harmful effects to human health. Further the inappropriate or indiscriminate use of disinfectants can develop bacterial resistance to antimicrobial agents.

5.6 Sanitary Survey and Questionnaires

Besides microbiological and physico-chemical analysis, reliable and more informative results may usually be obtained more quickly and cheaply from a 'Sanitary Survey' of an untreated water supply to check for defects such as leaks in well linings and pipes, the proximity of latrines, rubbish dumps, and other sources of pollution. The dissertant himself is resident of the study area. He has many relatives and he is familiar to each and every corner of the city as well. Therefore, it was easier for him during sanitary survey and questionnaires.

Though Patan has piped water supply, it offer relies on its traditional water supplies viz. dug wells, stone spouts etc. The piped system has never been sufficient to meet even the minimum demand of the people, besides acceptable water quality. The people of Kathmandu valley, on the whole, have become used to living with dry taps. Householders were forced to go back to the old wells and water spouts.

In Patan, there are hundreds of dug wells and many stone spouts from where the resi-

dents draw/collect water for their daily needs including drinking. Majority of the urban population depend on groundwater sources as the growing population uses more water than the reticulated system can supply. The validity of traditional water sources in urban Patan can be clearly understood from Plates 12 and 13. It is a matter of sad that several wells and spouts are in need of repair. The brick walls of many old wells were distorted and in deteriorating condition (Plate 6), and most of the aprons were found to be in poor condition and many of the drains leading away from them, ineffective.

Generally, the women collect water in the early morning between 5 and 8 a.m. and in the evening for drinking. They bring their own bucket and rope to fill their water vessels (*gagros*) from open wells. The water vessels which are called *gagros* contain 10-15 litres and are often carried on the hip by the women. Several trips may be made to collect water. Men come to the wells and spouts or shallow wells to wash themselves before the morning meal. Women come in the mid morning to wash clothes. Some people had very unhygienic habits of spitting, throwing sputum and phlegm around the water sources and wiping the hands on wearing clothes during water collection.

There was limited community consciousness about the environmental pollution and the resulting risk to health. Cleaning activities, in general, are looked upon as a very low status occupation. The persistence of traditional attitudes and traditional waste disposal habits of people are serious problems for effective management of environmental quality. In many places the children defaecate just outside the house and near to the water supplies. Unhygienic sewage disposal system and throwing wastes at random significantly contribute to the pollution of water resources. The sanitary condition of 48.6% sources was observed good, while rest 51.4% were in poor condition (Table 20). The survey of some confirmed aquifers supplying water to the spouts was also made. Nayekhyo Aquifer, Naricha Aquifer, Guita Aquifer, Khwayebahi Aquifer and Emu Dva Aquifer were all observed to be in unsanitary condition as open dump sites.

The exact figure of the average households dependent on each water source and total number of consumers could not be achieved because most of the sources were found to be crowded with people waiting for their turn to collect water. Residents from neighbourhood toles or localities were also observed to rush to the potential neighbourhood well or stone spout in dense inner area of the city.

Regarding the water pots, the use of pots made of brass was observed maximum, followed by aluminium and plastic ones. Very few people were found using copper pots and the use of steel and earthern pots were not observed at all (Table 22). People were found preferring aluminium and plastic pots instead of pots made of brass and copper. The interviewed people gave the reason as aluminium and plastic vessels are comparatively cheaper and lighter than pots made of brass and copper.

Nearly 85% of the people put cover on the drinking water storage vessels at home. However, they said that water vessels were often stored on the floor. Thus, children are likely to contaminate water with their hands when playing on the floor. The people often drink water without any kind of treatment at home. Only 0.8% of households drink boiled water, but some do it only when they are sick. Boiling of drinking water remains the most effective treatment, but interviewed people said that boiling of water consumes fuel and time, and boiled water is not tasty as well. Only 5.7% households possessed filter (Table 23).

This survey revealed that 33 wells and 1 spring were irregularly treated with bleaching powder by local residents (Table 23). The CDHP of the UMN has been working in urban Patan for 26 years in different sectors with an aim of making it a 'Healthy City'. The project has carried out the well chlorination programme to provide safe drinking water to the community people with the local participation. Lewis (1995) has reported-the well chlorination programme was successful in terms of awareness raising and community participation but has yet to prove itself in terms of the bacteriological quality of the water being treated. The target area of CDHP was limited and it has expanded activities in eight wards by 1997. It was learned from the survey that CDHP had given the duty of chlorination to one of the local resident with bleaching powder supplies in its target area. But, the chlorinating person was not doing the job reliably, either forgetting altogether or because of not getting regular bleaching powder supply (it was not known). In some wells pot chlorinator was observed, but the people informed that it had been 3-4 months or even more not applying the disinfectant in it. Some wells were treated with disinfectant only at the time of cleaning, according to the local residents. In some places people in non-target area and private well owners go to the Municipality for bleaching powder, but the application was irregular and unscientific as well. Such disinfection practice should be strictly discouraged since it can contribute to the development of resistance to the antimicrobial agents by bacteria.

Almost all interviewed people complained that they often suffer from common cold (flu) as a result of drinking water from groundwater sources. Diarrhoea, dysentery and typhoid cases were informed by 60.0%, 3.4% and 3.0% households respectively. Similarly, 16% said that children suffer from ascariasis. Average frequency of disorders in a family was 6-7 times per year (Table 24). During this study, 45% of the people expressed their unsatisfaction about the water quality commenting about the taste, while rest 55% said that wells and spouts were the only available water sources, and they have developed a habit. Potability of water was determined on the basis of clarity.

The study revealed an alarming picture of the existing water quality situation of groundwater supplies in Patan city. People are forced to consume highly polluted water from stone spouts and wells since water supply from centralized system is always inadequate. Outbreaks of waterbrone epidemics could occur at anytime. Therefore, preventive measures are urgently needed to control the occurrence of such unpleasureable incidence.

There is an inextricable relationship between bad sanitation and unhygienic practices, the consequential pollution of water supply and public health risk. Perhaps the most important factor apart from drinking water is sanitation and personal hygiene. During the sanitary survey it was noted that people often have little awareness about water contamination and its impact on health. For instance, the dissertant also made visit to some of the nearby houses around the sampling source just to observe whether people put cover on the water vessels or not, and the place where the water drawing bucket and rope for open wells were kept. In most of the cases, he observed unhygienic practices of storing the buckets and ropes on bare ground near the latrine before and after they are used for drawing water.

Covering the wells and fitting pumps would be contentious. Periodic cleaning of the open wells should be arranged to remove any accumulation of rubbish, sediment or vegetable growth, and disinfection before being put back into use. But, older residents had expressed the opinion that wells should be open to release bad gases, allow the sun in etc. Local people were reluctant and they had almost given up the tradition of cleaning the wells because of stories of deaths of people in wells in the last few years, possibly due to poisonous gas. Lewis (1995) also experienced the similar opinions of local residents in this regard.

The results of this study highlight the worsening situation on water availability, use and quality. It also warns that if the present trend of population growth continues in urban areas the time is not far to face severe water crisis even to live normal life. However, simple measures such as protection and conservation of water resources for improving both the output and quality of the water, sanitary improvement and hygienic practices will make able to meet the water demand thereby effectively reducing health risks. The use of ropes and buckets is a potential source of pollution where the well is an open one. This can be significantly minimized if communal ones are provided, so fixed to the wellhead that they cannot be removed, thrown on to the ground, or stored in an unsanitary manner. Further, paved or cemented well apron, pumps in good working order, clean surroundings of the water sources and good drainage help in water quality improvement. Above all, defaecation and washing clothes and people themselves within the immediate vicinity of the water sources must be forbidden/ prohibited.

Basic hygiene education programmes offer enormous positive effects in raising public consciousness about the risks of drinking contaminated water and in protecting society from waterborne diseases. In overall, the burden of water quantity and quality enhancement should be a shared responsibility among all users, concerned authorities and government. Concrete measures should be urgently taken for the protection and conservation of the irreplaceable groundwater sources.

CHAPTER - SIX

SUMMARY AND RECOMMENDATIONS

SUMMARY

The quality of water is of vital concern for mankind since it is directly linked with human welfare. All the waterborne diseases depend on faecal access to domestic water sources. However, the chain of transmission may be broken by safe disposal of wastes, hygienic practices as well as by protecting water supplies that are used as sources of drinking water.

Groundwater sources viz. wells, stone spouts and shallow pumps have been extensively used by the people in Kathmandu valley as the sources of drinking water from the ancient time. Majority of the population in urban Patan still depend on these sources. There have been a number of studies to assess the quality of the water. Almost all of these studies indicate faecal contamination of water sources in terms of bacterial quality. The results of this study also found most of the groundwater sources (68.6%) in urban area of Patan to exceed the WHO (1984) permissible level for coliforms, and posed a clear threat to public health. Different enteric bacteria including highly pathogenic ones were also isolated from 70% water samples in the present study. The values for some of the physico-chemical parameters for some samples found to lie above the maximum allowable limit set by WHO (1984), WHO (1993) and proposed standards by NWSC for drinking water.

High proportion (82.5%) of enteric bacteria isolates were found to be antibiotic resistant (AR) and multiple-antibiotic resistance (MAR) was expressed by more than half of the total isolates. The presence of AR bacteria, and particularly MAR bacteria in drinking water sources is a growing public health concern and it can have significant public health implications.

Some of the heavy metals, particularly silver exert toxic effect upon bacteria. In an attempt to study this effect silver, copper, brass, aluminium and steel were tested against some of the enterobacteria isolates in this study. The results showed silver and copper to be very effective, comparatively brass less effective, while aluminium and steel ineffective for their inhibitory effect against tested organisms. Thus, storage of drinking water in metallic pots that possess inhibitory effect can also purify the water.

The modern problems of environmental pollution are essentially the results of population explosion, unplanned urbanization and industrialization, improper or random disposal of solid wastes. Water pollution, today, has become a serious threat to mankind. Polluted and inadequate or unavailability of water, inadequate or poor sanitation, and unhygienic practices are responsible for all waterborne diseases. It is, therefore, high time to take necessary steps for the protection and conservation of water sources to improve both the output and quality of the water, and also to make the people aware of the magnitude, causes and social impact of the problem by the concerned authorities.

RECOMMENDATIONS

Following are some recommendations of the present study.

* Government, concerned agencies and local people should pay greater attention to the protection, preservation and maintenance of traditional water sources.
* Need of launching of environmental hygiene basic education programmes and activities to generate/raise public consciousness and support for the improvement of water quality and to widen health seeking behaviour among community people.
* The results of this study will be helpful in forecasting the possible outbreaks of waterborne diseases in the community.
* The situation being more critical, people should be educated about the simple and cheap techniques of water treatments such as boiling, filtering and chlorinating.
* Regular water quality monitoring should be carried out to check up the degree of pollution and its causes.
* Potential spouts after proper treatment can be used for the water re-distribution system (as proposed by Joshi *et al.*, 1992 for Alkva Hiti) to meet the acute shortage of water to the urban dwellers.
* The indiscriminate or inappropriate use of anti-microbials and their being taken at wrong dosages and for an insufficient length of time should be discouraged.
* Helpful in the choice and use of an appropriate water storage pots and filter types for better and safe drinking water.
* Lastly, the results could serve as a basis for the study of all future activities, planning and policy formulation related to water pollution.

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APPENDIX - I

Results of the features used in the Identification of Enterobacteria Isolates

Identified Organisms	Gram Nature	Cat	Ox	MR	VP	Ci	Ur	NO_3	OF	SIM			TSI			
										H_2S	Ind	M	Slope	Butt	H_2S	G
E. coli	-	+	-	+	-	-	-	+	F	-	+	+	Y	Y	-	+
E. coli, inactive	-	+	-	+	-	-	-	+	F	-	+	-	Y	Y	-	-
Escherichia blattae	-	+	-	+	-	+	-	+	F	-	-	-	Y	Y	-	+
Enterobacter aerogenes	-	+	-	-	+	+	-	+	F	-	-	+	Y	Y	-	+
Ent. agglomerans	-	+	-	-	+	+	-	+	F	-	-	+	Y	Y	-	-
Ent sakazakii	-	+	-	-	+	+	-	+	F	-	+	+	Y	Y	-	+
Citrobacter sp.	-	+	-	-	-	+	-	+	F	-	+	+	Y	Y	-	+
C. freundii	-	+	-	-	-	+	-	+	F	+	-	+	Y	Y	+	+
Klebsiella pneumoniae	-	+	-	-	+	+	+	+	F	-	-	-	Y	Y	-	+
K. oxytoca	-	+	-	-	+	+	+	+	F	-	+	-	Y	Y	-	+
Salmonella sp.	-	+	-	+	-	-	-	+	F	+	-	+	R	Y	+	+
S. Paratyphi A	-	+	-	+	-	-	-	+	F	-	-	+	R	Y	-	+
Shigella dysenteriae	-	+	-	+	-	-	-	+	F	-	+/-	-	R	Y	-	-
Morganella morganii	-	+	-	+	-	-	+	+	F	-	+	+	R	Y	-	+
Providencia alcalifaciens	-	+	-	+	-	+	-	+	F	-	+	+	R	Y	-	+
Serratia marcescens	-	+	-	+	+	+	-	+	F	-	-	+	R	Y	-	+
S. fonticola	-	+	-	+	-	+	-	+	F	-	-	+	R	Y	-	+/-
Hafnia alvei	-	+	-	+	+	-	-	+	F	-	-	+	R	Y	-	+
Yersinia enterocolitica	-	+	-	+	-	-	+	+	F	-	+/-	-	R	Y	-	-
Y. frederiksenii	-	+	-	+	-	-	+	+	F	-	+	-	R	Y	-	+/-
Y. kristensenii	-	+	-	+	-	-	+	+	F	-	+/-	-	R	Y	-	+/-
Y. intermedia	-	+	-	+	-	-	+	+	F	-	+	-	R	Y	-	-

Index :

Cat	- Catalase Test	NO_3	- Nitrate Reduction Test
Ox	- Oxidase Test	OF	- Oxidation-Fermentation Test
MR	- Methyl Red Test	H_2S	- Hydrogen Sulphide Production Test
VP	- Voges-Proskauer Test	Ind	- Indole Test
Ci	- Citrate Utilization Test	M	- Motility
Ur	- Urease Test	F	- Fermentative
Y	- Yellow (acid reaction)	R	- Red-Pink (alkaline reaction)
G	- Gas		

APPENDIX - II

Antibiotic Sensitivity Patterns of Enterobacteria Isolates

S.N.	Code	Organisms	Antibiotics									
			A	Ca	AK	G	C	Nf	T	Cf	Co	Na
1	$SS1_1$	*E. coli*	R	S	S	S	S	R	R	S	R	S
2	$SS1_2$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	S
3	$SS2_1$	*Salmonella sp.*	R	S	S	S	R	S	R	S	R	S
4	$SS2_2$	*E. coli*	R	S	S	S	R	S	R	S	R	S
5	$SS2_4$	*Y. enterocolitica*	R	S	S	S	S	R	S	S	S	S
6	$SS3_1$	*E. coli*	R	S	S	S	S	I	I	S	S	S
7	$SS3_2$	*K. pneumoniae*	R	S	S	S	R	R	R	R	R	R
8	$SS3_3$	*S. paratyphi A*	S	S	S	S	S	I	S	S	S	S
9	$SS3_4$	*Ent. sakazakii*	R	S	S	S	S	S	S	S	S	S
10	$SS7_1$	*Citrobacter sp.*	R	S	S	S	S	R	S	S	S	S
11	$SS7_2$	*Ent. aerogenes*	R	S	S	S	S	R	I	S	S	R
12	$SS7_3$	*S. paratyphi A*	R	S	S	S	S	R	R	S	S	S
13	$SS8_1$	*Sh. dysenteriae*	S	S	S	S	R	S	I	S	S	S
14	$SS8_2$	*Citrobacter sp.*	S	S	S	S	S	R	R	S	R	S
15	$SS8_3$	*E. coli, inactive*	R	S	S	S	S	R	R	S	R	R
16	$SS9_1$	*Ent. agglomerans*	R	S	S	S	S	R	R	S	S	S
17	$SS9_2$	*Ent. aerogenes*	R	S	S	S	S	R	I	S	S	S
18	$SS9_3$	*E. blattae*	S	S	S	S	S	I	S	S	S	S
19	$SS9_4$	*Y. frederiksenii*	S	S	S	S	S	S	S	S	S	I
20	$SS10_1$	*Y. frederiksenii*	S	S	S	S	S	R	R	S	R	S
21	$SS10_2$	*S. marcescens*	R	S	S	S	R	R	R	S	S	S
22	$SS10_3$	*S. paratyphi A*	S	S	S	S	S	I	I	S	S	S
23	$SS10_4$	*Sh. dysenteriae*	S	S	S	S	S	R	S	S	S	S
24	$SS11_1$	*C. freundii*	S	S	S	S	S	R	R	S	S	S
25	$SS11_2$	*Ent. aerogenes*	R	S	S	S	S	R	I	S	S	S
26	$SS11_3$	*Y. enterocolitica*	R	S	S	S	S	R	S	S	S	S
27	$SS12_1$	*E. coli*	S	S	S	S	S	I	R	S	S	S
28	$SS12_2$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	R
29	$SS12_3$	*C. freundii*	R	S	S	S	R	R	R	S	I	I
30	$SS13_1$	*S. marcescens*	R	S	S	S	R	R	R	S	S	S

INDEX :

A	Ampicillin	Nf	Nitrofurantoin
Ca	Ceftazidime	T	Tetracycline
Ak	Amikacin	Cf	Ciprofloxacin
G	Gentamycin	Co	Co-trimoxazole
C	Chloramphenicol	Na	Nalidixic Acid

S Sensitive I Intermediate Resistant R Resistant

Antibiotic Sensitivity Patterns of Enterobacteria Isolates

S.N.	Code	Organisms	Antibiotics									
			A	Ca	AK	G	C	Nf	T	Cf	Co	Na
31.	SS13$_2$	*Sh. dysenteriae*	I	S	S	S	R	R	S	S	S	S
32.	SS13$_3$	*Ent. agglomerans*	S	S	S	S	S	S	S	S	S	S
33.	SS13$_4$	*Y. enterocolitica*	R	S	S	S	S	R	S	S	S	S
34.	SS14$_1$	*Hafnia alvei*	R	S	S	S	R	S	R	S	S	S
35.	SS14$_2$	*E. coli*	S	S	S	S	S	I	S	S	S	S
36.	SS14$_3$	*S. marcescens*	R	S	S	S	R	R	R	S	S	S
37.	W01$_1$	*Ent. agglomerans*	S	S	S	S	R	R	R	S	S	S
38.	W01$_2$	*C. freundii*	S	R	S	S	S	R	S	S	S	S
39.	W02$_1$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	S
40.	W02$_2$	*C. freundii*	S	S	S	S	S	R	I	S	S	S
41.	W03$_1$	*H. alvei*	S	S	S	S	S	R	S	S	S	S
42.	W03$_2$	*Ent. aerogenes*	S	S	S	S	S	I	S	S	S	S
43.	W03$_3$	*Ent. agglomerans*	S	S	S	S	S	I	S	S	S	S
44.	W04$_1$	*Ent. aerogenes*	R	S	S	S	R	R	S	S	I	S
45.	W05$_1$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	S
46.	W05$_2$	*E. coli*	I	S	S	S	S	I	R	S	S	S
47.	W05$_3$	*H. alvei*	R	S	S	S	S	S	R	S	S	S
48.	W06$_1$	*Ent. aerogenes*	S	S	S	S	S	R	R	S	S	S
49.	W07$_1$	*Ent. agglomerans*	S	S	S	S	S	S	I	S	S	S
50.	W08$_1$	*E. coli*	S	S	S	S	S	R	S	S	S	S
51.	W08$_2$	*Sh. dysenteriae*	S	S	S	S	S	R	S	S	S	S
52.	W09$_1$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	S
53.	W09$_2$	*E. coli*	S	S	S	S	S	R	S	S	S	S
54.	W09$_3$	*C. freundii*	S	R	S	S	S	R	S	S	S	S
55.	W011$_1$	*Serratia fonticola*	S	S	S	S	R	R	S	S	S	R
56.	W011$_2$	*Ent. agglomerans*	S	S	S	S	S	I	S	S	I	S
57.	W011$_3$	*S. paratyphi A*	R	S	S	S	S	S	S	S	S	S
58.	W011$_4$	*C. freundii*	I	S	S	S	R	R	R	S	S	S
59.	W012$_1$	*Salmonella sp.*	I	S	S	S	S	R	I	S	S	S
60.	W012$_2$	*Ent. agglomerans*	S	S	S	S	S	R	R	S	S	S

INDEX :

A = Ampicillin
Ca = Ceftazidime
Ak = Amikacin
G = Gentamycin
C = Chloramphenicol
Nf = Nitrofurantoin
T = Tetracycline
Cf = Ciprofloxacin
Co = Co-trimoxazole
Na = Nalidixic Acid

S = Sensitive
I = Intermediate Resistant
R = Resistant

Antibiotic Sensitivity Patterns of Enterobacteria Isolates

S.N.	Code	Organisms	Antibiotics									
			A	Ca	AK	G	C	Nf	T	Cf	Co	Na
61	$W012_1$	*S. paratyphi A*	S	S	S	S	S	R	S	S	S	S
62	$W013_1$	*Ent. aerogenes*	S	S	S	S	S	S	I	S	S	S
63	$W013_2$	*C. freundii*	R	R	S	S	R	R	R	S	S	S
64	$W016_1$	*Citrobacter sp.*	R	S	S	S	S	R	S	S	R	S
65	$W016_2$	*H. alvei*	R	S	S	S	S	R	R	S	R	S
66	$W017_1$	*C. freundii*	R	R	S	S	R	R	I	S	S	I
67	$W017_2$	*Salmonella sp.*	S	S	S	S	S	R	S	S	S	S
68	$W017_3$	*Sh. desenteriae*	R	S	S	S	S	R	S	S	R	S
69	$W017_4$	*Ent. agglomerans*	R	S	S	S	R	R	S	S	S	S
70	$W018_1$	*Ent. agglomerans*	R	S	S	S	S	R	I	S	S	S
71	$W018_2$	*E. coli*	S	S	S	S	S	R	I	S	S	S
72	$W019_1$	*Sh. desenteriae*	I	S	S	S	S	S	S	S	S	S
73	$W019_2$	*E. coli*	R	S	S	S	S	S	S	S	S	S
74	$W019_3$	*E. coli, inactive*	R	S	S	S	S	R	R	S	R	S
75	$W020_1$	*E. coli*	S	S	S	S	S	R	S	S	R	S
76	$W020_2$	*S. paratyphi A*	R	S	S	S	S	R	S	S	S	S
77	$W020_3$	*Ent. aerogenes*	R	S	S	S	S	R	I	S	S	S
78	$W020_4$	*C. freundii*	S	R	S	S	S	R	S	S	S	S
79	$W021_1$	*H. alvei*	S	S	S	S	S	R	S	S	S	S
80	$W022_1$	*Ent. agglomerans*	S	S	S	S	S	I	S	S	S	S
81	$W022_2$	*E. coli*	S	S	S	S	S	I	I	S	S	S
82	$W024_1$	*E. coli*	R	S	S	S	S	I	S	S	S	S
83	$W024_2$	*Ent. agglomerans*	R	S	S	S	S	S	S	S	S	S
84	$W024_3$	*H. alvei*	R	S	S	S	S	R	S	S	S	S
85	$W025_1$	*E. coli*	S	S	S	S	S	R	S	S	S	S
86	$W026_1$	*Ent. aerogenes*	S	S	S	S	S	R	R	S	S	S
87	$W026_2$	*Y. intermedia*	S	S	S	S	S	R	S	S	S	S
88	$W026_3$	*H. alvei*	S	S	S	S	S	R	R	S	S	S
89	$W028_1$	*Ent. aerogenes*	R	S	S	S	R	R	I	S	S	S
90	$W028_2$	*Y. enterocolitica*	R	S	S	S	S	R	R	S	R	S

INDEX :

A = Ampicillin
Ca = Ceftazidime
Ak = Amikacin
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C = Chloramphenicol
Nf = Nitrofurantoin
T = Tetracycline
Cf = Ciprofloxacin
Co = Co-trimoxazole
Na = Nalidixic Acid

S = Sensitive
I = Intermediate Resistant
R = Resistant

Antibiotic Sensitivity Patterns of Enterobacteria Isolates

S.N.	Code	Organisms	Antibiotics									
			A	Ca	AK	G	C	Nf	T	Cf	Co	Na
91.	$W028_3$	*E. coli*	R	S	S	S	S	S	S	S	S	S
92.	$WC2_1$	*E. coli*	S	S	S	S	S	S	S	S	S	S
93.	$WC2_2$	*K. oxytoca*	R	S	S	S	S	R	I	S	S	S
94.	$WC3_1$	*E. coli*	S	S	S	S	S	I	I	S	S	S
95.	$WC3_3$	*Providencia alcalifaciens*	R	S	S	S	S	R	S	S	S	S
96.	$WC5_1$	*Salmonella sp.*	S	S	S	S	S	R	I	S	S	S
97.	$WC7_1$	*E. blattae*	S	S	S	S	S	I	I	S	S	S
98.	$WC7_2$	*Ent. aerogenes*	S	S	S	S	S	R	I	S	S	S
99.	$WC7_3$	*H. alvei*	R	S	S	S	S	S	R	S	S	S
100.	$WC8_1$	*Y. kristensenii*	S	S	S	S	S	R	S	S	S	S
101.	$WC8_2$	*Morganella morganii*	R	S	S	S	S	R	S	S	S	S
102.	$WC9_1$	*Ent. aerogenes*	S	S	S	S	R	I	I	S	S	S
103.	$WC13_1$	*C. freundii*	R	S	S	S	R	R	R	S	S	S
104.	$WC15_1$	*Salmonella sp.*	S	S	S	S	S	R	I	S	S	S
105.	$WC15_2$	*Citrobacter sp.*	R	S	S	S	S	R	S	S	S	S
106.	$WC16_1$	*Ent. agglomerans*	S	S	S	S	S	I	S	S	S	S
107.	$WC16_2$	*Salmonella sp.*	S	S	S	S	S	R	I	S	S	S
108.	$WC17_1$	*Ent. aerogenes*	R	S	S	S	S	R	R	S	S	S
109.	$WC18_1$	*Salmonella sp.*	S	S	S	S	R	R	R	S	S	S
110.	$WC18_2$	*E. coli*	I	S	S	S	S	I	I	S	S	S
111.	$WC18_3$	*H. alvei*	R	S	S	S	S	R	S	S	S	S
112.	$WC19_1$	*Ent. aerogenes*	R	S	S	S	S	R	I	S	S	S
113.	$WC19_2$	*Ent. agglomerans*	R	S	S	S	S	I	S	S	S	S
114.	$SW1_1$	*E. coli*	R	S	S	S	R	R	S	S	S	S
115.	$SW1_2$	*Ent. aerogenes*	S	S	S	S	R	R	I	S	S	R
116.	$SW1_3$	*Ent. agglomerans*	S	S	S	S	S	I	S	S	S	S
117.	$SW3_1$	*E. coli*	S	S	S	S	S	R	R	S	R	S
118.	$SW2_1$	*Ent. aerogenes*	S	S	S	S	S	R	R	S	S	S
119.	$SW2_2$	*Citrobacter sp.*	R	S	S	S	S	R	S	S	S	S
120.	$SW2_3$	*E. coli*	S	S	S	S	S	I	S	S	S	S

INDEX :

A — Ampicillin
Ca — Ceftazidime
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Co — Co-trimoxazole
Na — Nalidixic Acid

S — Sensitive
I — Intermediate Resistant
R — Resistant

APPENDIX - III

I. **Composition and Preparation of Media Used in Isolation and Identification of Bacteria.**

(All compositions are given in grams per litre and pH at 25°C temperature.)

(i) **CULTURE MEDIA**

1. **Nutrient Agar (NA)**

Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15.0
Final pH	7.4 ± 0.2

2.8 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

2. **Nutrient Broth (NB)**

Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final pH	7.4 ± 0.2

1.3 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

3. **Selenite F Broth**

Tryptone	5.0
Lactose	4.0
Sodium Phosphate	10.0
Sodium Acid Selenite	4.0
Final pH	7.4 ± 0.2

2.3 gms of media was dissolved in 100 ml of distilled water and heated to dissolve the media and then sterilized in a boiling water bath for 10 minutes. The media was not autoclaved and excessive heating was avoided.

4. **Salmonella-Shigella (S-S) Agar**

Beef Extract	5.0
Peptone	5.0
Lactose	10.0
Bile Salt	8.5
Sodium Citrate	10.0
Sodium Thiosulphate	8.5
Ferric Citrate	1.0
Brilliant Green	0.00033
Neutral Red	0.025
Agar	15.0
Final pH	7.0 ± 0.2

6.3 gms of media was dissolved in 100 ml of distilled water and heated with frequent agitation to dissolve the media completely. The media was not autoclaved and over heating was avoided.

5. **Eosin Methylene Blue (EMB) Agar**

Peptone	10.0
Lactose	10.0
Dipotassium Hydrogen Phosphate	2.0
Agar	15.0
Methylene Blue	0.065
Eosin Y	0.4
Final pH	7.1

36 gms of media was dissolved in 1000 ml distilled water and heated to dissolve the media completely. It was cooled to 50°C and shaken in order to oxidise the methylene blue. The media was sterilized at 15 lbs at 121°C for 15 minutes.

6 **M-Endo Agar**

Casein Enzymic Hydrolysate	3.7
Peptic Digest of Animal Tissue	3.7
Tryptose	7.5
Yeast Extract	1.2
Lactose	9.4
Dipotassium Phosphate	3.3
Monopotassium Phosphate	1.0
Sodium Chloride	3.7
Sodium Deoxycholate	0.1
Sodium Laurylsulphate	0.05
Sodium Sulphite	1.6
Basic Fuchsin	0.8
Agar	15.0
Final pH	7.2 ± 0.2

5.1 grams of media was dissolved in 100ml distilled water containing 2ml of 95% ethanol. It was boiled to dissolved the medium completely. It was cooled to 45°C and poured in petriplates.

Note : The medium was not autoclaved.

7 **M- FC Agar**

Tryptose	10.0
Proteose Peptone	5.0
Yeast Extract	3.0
Lactose	12.5
Bile Salts Mixture	1.5
Sodium Chloride	5.0
Aniline Blue	0.1
Agar	15.0
Final pH	7.4 ± 0.2

5.2 grams of media was dissolved in 99ml of distilled water. One ml of reconstituted contents of Rosalic acid (FD058) was added. It was boiled to dissolve the medium completely. The medium was cooled to 45°C and poured in petriplates

Note : The medium was not autoclaved.

8. **Xylose Lysine Deoxycholate (XLD) Agar**

Xylose	3.5
L - Lysine	5.0
Lactose	7.5
Sucrose	7.5
Sodium Chloride	5.0
Yeast Extract	3.0
Sodium Deoxycholate	2.5
Sodium Thiosulphate	6.8
Ferric Ammonium Citrate	0.8
Phenol Red	0.08
Agar	15.0
Final pH	7.4 ± 0.2

5.65 grams of media was dissolved in 100ml of distilled water. It was heated with frequent agitation, but overheating was avoided. The medium was transferred immediately to a water bath at 50°C. After cooling, it was poured into sterile petriplates.

II. Composition and Preparation of Media Used for Antibiotic Sensitivity Test.

Mueller Hinton Agar (MHA)

Beef Infusion Broth	300.0
Casein Acid Hydrolysate	17.0
Starch	1.0
Agar	17.0
Final pH	7.0 + 0.2

III. BIOCHEMICAL MEDIA AND BIOCHEMICAL TESTS

MR - VP media

Bufferred Peptone	7.0
Dextrose	5.0

Dipotassium Phosphate	5.0
Final pH	6.9 ±0

1.7 gm of MR-VP powder was weighed and dissolved in 100ml of distilled water. About 10ml amounts were distributed in several test tubes and sterilized by autoclaving at 121°C at 15 lbs for 15 minutes.

Methyl Red Test

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red colour with the indicator methyl red.

Procedure

The test was performed by inoculating a colony of the test organism in 0.5 ml of sterile glucose phosphate broth, and it was incubated at 37°C for 48 hours. Then a drop of methyl red reagent was added. A bright red colour indicates positive test.

Voges - Proskauer (V-P) Test

This test is employed to detect the production of acetyl methyl carbinol or its reduction product 2,3 butylene glycol during fermentation of carbohydrates.

Procedure

Culturing process is same as in methyl red test. Barritt reagent was added to the 2ml of 48 hrs old culture. A positive reaction is indicated by the development of a pink colour within 30 minutes. The tube can be shaken at intervals to ensure maximum aeration.

Sulfide - Indole - Motility (SIM) Medium

Beef extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	0.30
Final pH	7.3±0.2

3.6 gms of media was dissolved in 100ml of distilled water and heated to boil. Then the

medium was distributed in tubes to a depth of about 3 inches. Sterilization was done in autoclave for 15 minutes at 15 lbs at 121°C.

Use

This medium (SIM) is used for the determination of sulphide production, indole formation and motility of enteric bacteria.

Procedure

SIM media was inoculated with a pure culture of organism by inserting a straight wire to about one third of the depth of the medium and incubated at 37° for 18-24 hours. The medium was examined for motility and H_2S production at first and then indole production.

Indole Test

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

Citrate Utilization Test

This test is performed to detect whether an organism utilizes citrate as its only source of carbon, and ammonia as its only source of nitrogen or not by using simmon's citrate agar.

Simmon's Citrate Agar

Magnesium Sulphate	0.2
Monoammoium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Bromo Thymol Blue	0.08
Agar	15.0
Final pH	6.8 ±0.2

2.42 gms of media was dissolved in 100ml of distilled water and heated to boil. The medium was distributed in tubes and sterilized by autoclaving at 121°C at 15 lbs. for 15 minutes.

Procedure

Simmon's citrate agar tube slants were inoculated with test organisms by streaking with straight wire. Citrate utilization is indicated by growth and colour change of medium form green to bright blue.

Triple Sugar Iron (TSI) Agar

Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
Final pH	7.4 ± 0.2

6.5 grams of media was dissolved in 100ml of distilled water and boiled to dissolve completely. It was distributed in tubes and sterilized by autoclaving at 15 lbs at 121°C for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch long.

Use

TSI agar is used 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 production.

Urea Broth Base

Monopotassium Phosphate	9.1
Dipotassium Phosphate	9.5
Yeast Extract	0.1

Phenol Red	0.01
Final pH	6.8 ± 0.2

1.85 gramsof media was dissolved in 95 ml of distilled water. It was seterilized by autoclaving at 15 lbs at 121°C for 15 mins. It was then cooled to 55°C, and 5ml of sterile 40% urea solution (FD048) was aseptically added. The contents were mixed well and distributed into sterile test tubes.

Hugh and Leifson Medium

Peptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Agar	2.0
Bromo Thymol Blue	0.05
Glucose	10.0
Final pH	7.1 + 0.2

1.93 grams of media was dissolved in 100ml of distilled water. It was boiled to dissolve the medium completely and distributed into tubes in duplicate. The medium was then sterilized by autoclaving at 15 lbs at 121°C for 15 minutes.

Note : *All the media and antibiotic disks used in this study were the products of Hi-Media Laboratories Pvt. Limited, Bombay - 400 086, India.*

APPENDIX - IV

Composition 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 with distilled water. Then, it was flooded with decolorizer acetone and immediately washed with water. The smear was treated with safranin for 1 minute and again washed with distilled water. It was dried and observed under microscope.

Preparation of Stains

i. **Crystal Violet**

Solution A

Crystal Violet	2.0 gm
95% ethyl alcohol	20.0 ml

Solution B

Ammonium oxalate	0.8 gm
Distilled water	30.0 ml

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 iodide	2.0 ml
Distilled water	300.0 ml

Iodine and potassium iodide 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. **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.

Concentrated Hydrogen Peroxide	3.0 ml
Distilled water	97.0 ml

Procedure

Three ml of 3% hydrogen peroxide was taken in a test tube and a 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 prodution of gas bubbles immediately indicates positive catalase test.

3. **Oxidase Test**

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

Reagent: 1 % solution of tetramethyl-þ-phenylene-diamine dihydrochloride

To make 10 ml :

Tetramethyl-þ-Phenylenediamine	0.1
Distilled Water	10.0

Whatman 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 up using glass rod and rubbed to the paper. Development of violet colour within 10 seconds is an indication of positive test.

APPENDIX - V

SURVEY OF GROUNDWATER SOURCES AND QUESTIONNAIRES WITH CONSUMERS IN AN URBAN AREA OF PATAN

A. *Sampling Report*

1.0 Sampling Site : ..... Ward No : .....

2.0 Sampling Date ..... Sampling Time : ..... Code No. : .....

3.0 Source : Well ☐ Tube-Well (Shallow Pump) ☐ Stone Spout ☐ Shallow Well ☐

If dug well or shallow well,

(a) Depth ..... m (Deep ☐ / Shallow ☐)

(b) Open ☐ / Covered ☐

3.1 Recently Constructed ☐ / Old ☐

3.2 In Use ☐ / Not in Use ☐

4.0 Temp. of the source of Sample ..... °C pH : .....

5.0 Sanitary condition of the sampling site .....

.....

5.1 Possible sources of pollution (if any) & their approximate distance from the sampling point :

.....

.....

5.1 Open Latrine ☐ / Garbage ☐

B. *Questionnaires with Consumers*

1.0 *Used for* Drinking Only☐ Washing Only☐ Washing and Bathing☐ For all purpose ☐

(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

2.0 *No. of households dependent on water source* : .....

2.1 *No. of family members* :

(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

2.2 *Total number of consumers* .....

3.0 **Treatment (if any) :** Yes ☐ No ☐ Sometimes ☐

3.1 *Community level treatment at the source* : Yes ☐ No ☐

If Yes Bleaching Powder☐ Chlorination ☐ Potash ☐ Alums ☐ Others ☐

3.2 *Domestic level treatment (for drinking)* :

Boiling :

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

Use of Filter :

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

Type of Filter :

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

Using Potash

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

3.2.1 *Why do you not boil water for drinking ?*

a) Because it consumes fuel

b) Because it is time consuming

c) Both (a) and (b)

d) Because boiled water is not tasty

e) All of the above

f) No need of boiling since water is potable

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

4.0 *Pot type used for drinking water collection and storage* at the home :

Clay ☐ Aluminium ☐ Brass ☐ Copper ☐ Steel ☐ Plastic ☐

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

4.1 *Do you put cover on the drinking water storage vessels at home ?* Yes ☐ No ☐

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

5.0 *Do you have tap at home* Yes ☐ No ☐

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

5.1 *In your opinion is the municipal water supply clean and safe for drinking ?* Yes ☐ No ☐ Sometimes dirty ☐

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

6.0 *Reasons for using groundwater source* :

(a) No sufficient municipal water supply

(b) No tap (municipal supply) at home

(c) Water quality is better than that of municipal supply

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

6.1 *Dependence on water source for drinking* :

(a) Only when tap turns dry
(b) Most often
(c) Throughout the year
(d) Not at all

(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

6.2 *Comments of users on existing water quality* :

.....

.....

Satisfactory ☐ Unsatisfactory ☐

(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

7.0 *Do you clean the well ?* Yes ☐ No ☐

If Yes, when , how and frequency ? .....

8.0 **Occurrence of Waterborne Diseases**

S.N.	Yes / No	Age Group (Children/Adult)	Frequency in a family/year	Type of disorder(s)

(a)Diarrhoea (b)Dysentery (c)Jaundice (d)Typhoid (e)Pneumonia (f)Ascariasis (g)Don't know

9.0 *What do you think is the main cause for such disorders* ?

(a) Dirty water
(b) Lack of cleanliness
(c) Negligence on personal hygiene
(d) All of the above

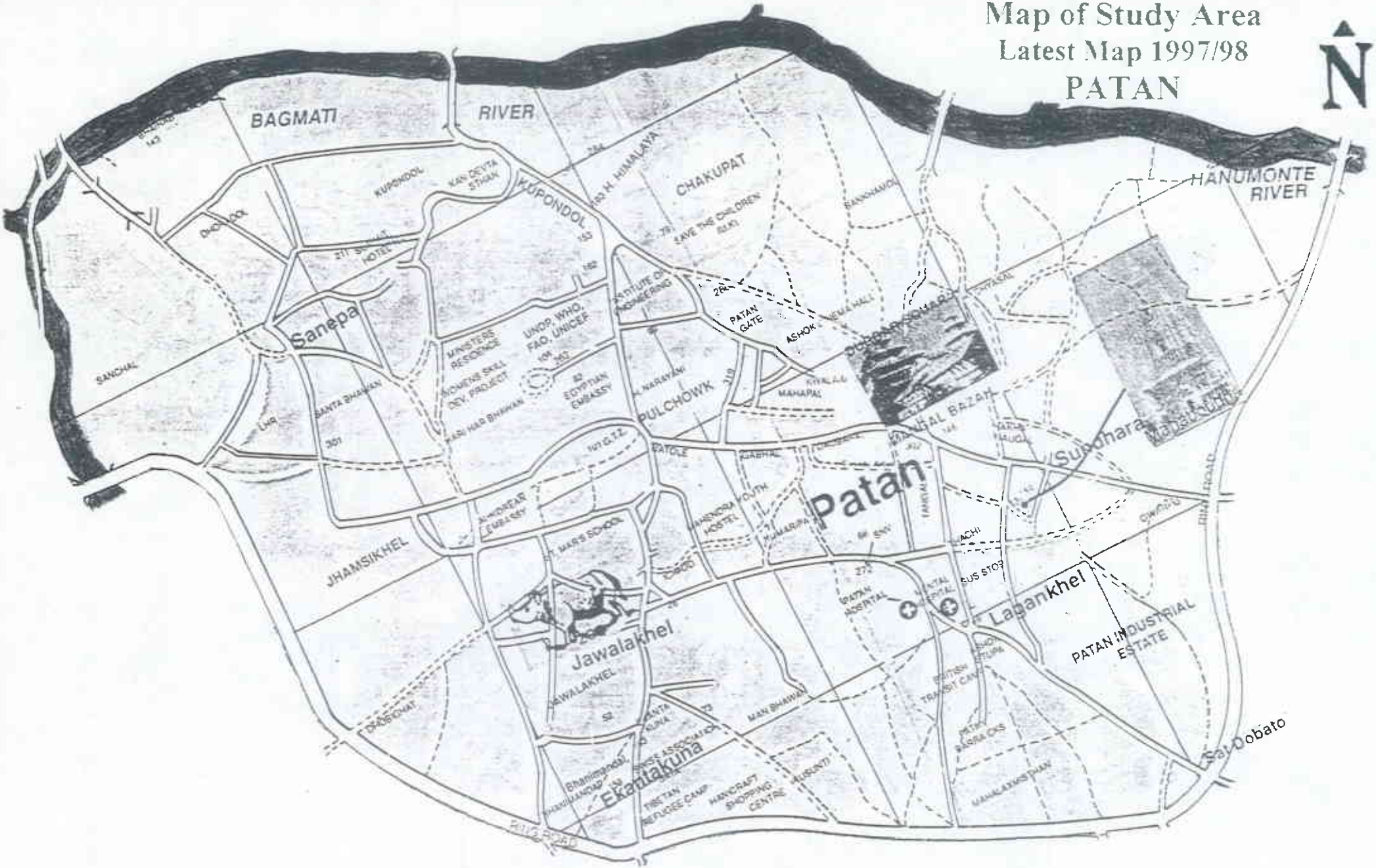
(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

10.0 *In your opinion, the main cause that can pollute groundwater supply is* :

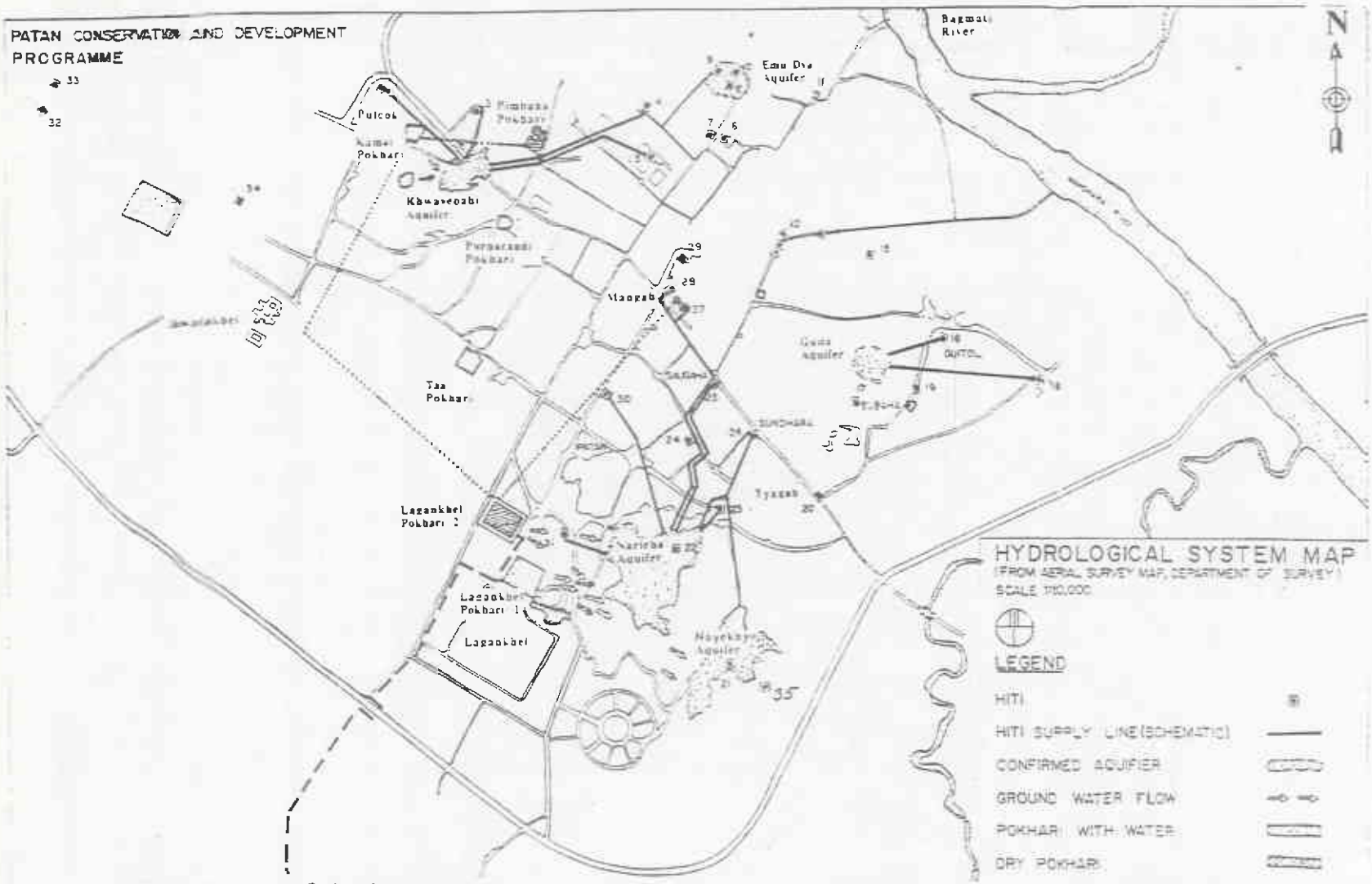
(a) Dirts surrounding the source
(b) Habit of open disposal of refuses
(c) Lack of proper sewage and sanitation system
(d) Broken and leakage of sewage pipelines, septic tank leachate
(e) Children pollute the source
(f) All of the above
(g) I don't know about it

(1) ..... (2) ..... (3) ..... (4) ..... (5) ..... (6) ..... (7) ..... (8) ..... (9) ..... (10) .....

Map of Study Area
Latest Map 1997/98
PATAN



Location Map of Stone Spouts



1. Pulchok Hiti
2. Gain Hiti
3. Cawa Hiti
4. Tapah Hiti
5. Nagbaha Hiti
6. Misa Hiti
7. Konti Hiti
8. Amrit Hiti
9. Alkva Hiti
10. Wasah Hiti
11. Sainthu Ganesh Hiti
12. Chyasah Hiti
13. Nay Hiti
14. Bya Hiti
15. Bhole Hiti
16. Makah Hiti
17. Subaha Hiti
18. Balkumari Hiti
19. Guita Hiti
20. Tyagah Hiti
21. Sinci Hiti
22. Nah Hiti
23. Kanibaha Hiti
24. Thapah Hiti
25. Saugah Hiti
26. Lun Hiti
27 Loh Hiti
28. Thusa Hiti
29. Mangah Hiti
30. Tangah Hiti
31. Loh Hiti
32. Iku Hiti
33. Hiku Hiti
34. Jawalakhyo Hiti
35. Manah Hiti