"WATERBORNE DISEASES IN SHIMLA CITY: AN ANALYSIS OF WATER SUPPLY SCHEME"

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Dr. RAJIV GANGULY
(Associate Professor)

By

VIPASHA SHARMA
(152760)

to

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

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CERTIFICATE

This is certify that work which is being presented in the thesis entitle **"WATERBORNE DISEASE IN SHIMLA:AN ANALYSIS OF WATER SUPPLY SCHEME"** in partial fulfillment of the requirement for the award of the degree of Master of technology in Civil Engineering with specialization in **"Environmental Engineering"** and submitted in Department of Civil Engineering, **Jaypee University of Information Technology, Waknaghat** is an authentic record of work carried out by **Vipasha Sharma** during a period from July, 2016 to May, 2017 under the supervision of **Dr. Rajiv Ganguly**, Associate Professor, Department of Civil Engineering, Jaypee University of Information Technology, Waknaghat, Solan.

The above statement made is correct to the best of our knowledge.

Date: 2/5/17

Dr. Ashok Kumar Gupta
Professor& Head of Department
Department of Civil Engineering
JUIT Waknaghat

Rganguly 02/05/17
Dr. Rajiv Ganguly
Associate Professor
Department of Civil Engineering
JUIT Waknaghat

External Examiner

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ABSTRACT

Shimla is a beautiful city and a rapidly growing hill station, attracting a large number of people from various parts of the world since British period. Sudden increase in population has triggered many problems like water shortage, poor sewage network and lack of quality drinking water. Outbreaks regarding waterborne disease are common in densely populated and water-scarce cities like Shimla. Mass level Jaundice was reported in Shimla City during 2015-16 affecting about 1680 people within 3 months which was of great concern. Cases peaked in January 2016 and decreased rapidly, suggesting a common source outbreak. Through studies it was found three major zones supplied by a common water supply i.e., Ashwani Khud was severely affected. The water after being pumped from the source was treated using conventional water treatment process. Since branch of this stream was receiving treated sewage effluent from a nearby Sewage Treatment Plant, which might have increased the bacterial load of the source water and thus leading to the outbreak. The efficiency of WTP and Water quality of AshwaniKhad water supply system was analyzed to check it's suitability for drinking purposes.

The objectives of the investigation were to (i) Determination of the cause of problem and population at risk. (ii) Assessment of water quality variables in drinking water sources, Water Treatment Plant and Distribution Systems. (iii) Determine the degree of contamination at different stages of water supply. (iv) Identification of Pathogens in Drinking Water Supplies.

The present study was aimed to identify pathogens in the water supply. For this, various bacteriological tests like colony forming units, MPN, selective isolation of bacteria and biochemical tests were performed. The water samples were found negative for any sort of fecal contamination. Biochemical tests lead to the presence of *Alcaligenes faecalis, Pseudomonas* spp., *Streptococcus* spp.in the water samples. All of the species discovered showed persistence in water supply and distribution system which can be a result of lack of proper disinfection process and maintenance of piped systems.

***Keywords*:** Waterborne Pathogens and Disease(s), Drinking Water, AshwaniKhad, Colony forming units (CFU), Most probable number (MPN), Bacteria.

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List of Symbols

WTP	Water Treatment Plant
RC	Residual Chlorine
WHO	World Health Organizations
LWSS	Low Water Supply Scheme
I&PH	Irrigation and Public Health
MR	Methyl Red
VP	Voges-Proskauer
Ig	Immunoglobulin
XLD	Xylose-Lysine Deoxycholate
TCBS	Thiosulphate Citrate bile salts
TSI	Triple sugar iron agar
MPN	Most probable Number
CFU	Colony Forming Units
MLD	Million liters per day
LFC	Lactose fermenting colonies
NLFC	Non-lactose fermenting colonies
WC	White colonies
TSC	Transparent small colonies
T	Temperature
°	Degrees
µ	micro

CHAPTER: 01

INTRODUCTION

1.1 General

This chapter briefly introduces the research topic and the need for study. It also provides the description about the importance of effective water treatment systems and its role in prevention of waterborne disease. It also includes the outline of the project and objectives.

1.2 Water and waterborne disease

Water is one of the basic necessities of all living organisms and access to safe drinking water is a basic human right and essential need as per health perspective. In spite of water being a renewable resource majority of population is living under scarcity of water. Due to the increase in population the demand of water is increasing, creating tremendous pressure on existing water sources, while improvement in the living standards of the society is leading to deterioration of water quality. In recent times, due to advancement in technologies, the demand for water has roseexponentially to meet the industrial, agricultural, and domestic activities. The various types of industrial and municipal wastes are dumped in rivers with little or hardly any treatment, thus, increasing the presence of toxic organic and inorganic substances in water and waste water, resulting in waterborne diseases.

The effect of poor water quality on human health is enormous. It is estimated that about 37.7 million Indians are affected by waterborne diseases annually while 1.5 million children are estimated to die of diarrhea alone (Water Aid, 2005, NCDC).

Contaminants of drinking water can be physical, chemical, and biological (Palamuleni, 2002).The entry of contaminants into the water distribution network can occur via storage tanks (by animals, dust-carrying bacteria, and infiltration) and pipes. Thus, need is felt for a sensor network that could help identify the locationof the source, concentration of contamination and the time of occurrence. (Tao et al.)

Waterborne diseases can be a result of contamination of water with virus (Viral hepatitis, Poliomyelitis), bacteria (typhoid,cholera,dysentery), parasites (worm infestation, guinea wormsetc.), or chemical contamination of water by reckless discharge of various industrial effluents in the drinking water sources.

1.2.1 Waterborne pathogens

The probabilities of contamination from feces of human and animal origin pose greatest threat to drinking water supplies. Table 1.1 shows the various types of waterborne pathogens, their source and symptoms if ingested by human beings.

Table 1.1 Types of waterborne pathogens and disease caused by them.

Disease and microorganism responsible	Sources of Agent in Water Supply	General Symptoms
PROTOZOA		
Cryptosporidiosis (oral)	Filtrates on water filters and membranes, runoff of water from agricultural lands.	Symptoms can be like flu, watery discharge of stools, loss of weightloss of appetite, or nausea.
Giardiasis (fecal-oral)	Untreated water, campgrounds where humans and wildlife use same source of water.	Diarrhea, abdominal discomfort, bloatingand flatulence.
PARASITES		
Echinococcosis (Hydatid disease)	contaminated water containing feces	Liver enlargement, in many cases cysts rupture can cause anaphylactic shock.
Ascariasis	Contamination of water by human/animal feces	Disease is asymptomatic or accompanied by fever, diarrhea, vomiting, and nausea.
BACTERIA		
Campylobacteriosis	Drinking water contaminated with feces	Produces dysentery like symptoms along with a high fever.
Dysentery	Water contaminated with the bacterium	Passage of feces with blood or mucus and vomiting of blood.
Salmonellosis	Ingestion of water contaminated with feces of an infected person	Sustained fever up to 40 °C and diarrhea.Worse condition may cause spleen and liver enlargement if untreated may even cause death.
VIRUS		
Hepatitis A (HEPATOVIRUS)	Can manifest itself in water (and food)	Symptoms are only acute and include Fatigue, fever, abdominal pain, nausea, diarrhea, jaundice and depression.

Hepatitis E (HEPATOVIRUS)	Contaminated drinking water	Self-limiting, few cases may develop into acute liver failure, fever, nausea and vomiting, abdominal pain. Jaundice with dark urine and pale stools and an enlarged, tender liver.

1.2.2 Water and its significance to public health

Sanitation and safe drinking water are fundamental rights for the basic health of humans. Worldwide, it is reported, merely two in ten percent population do not have access to safe drinking water; and four of every ten percent population, do not even have access to simple latrine (Bartram et al., 2005). This condition is even worse in the developing countries where one in three people lack safe drinking water and sanitation. In such countries, the consumption of contaminated water led to more than 80% of diseases and one third of the total deaths (Palamuleni, 2002). About half the population in developing countries, is suffering from diseases associated with water supply and sanitation at the same time, which may lead to waterborne diseases such as diarrhea, malaria, cholera and other water related disease (Ashbolt, 2004; WHO, 2005) thus, purity of drinking water conforming to prescribed standards(WHO,APHA) should be of primary concern of any water supply system. According to IDSP, diarrheal cases can be reduced by improving water supply and sanitation.Many of these diseases are directly or indirectly related to lack of safe drinking waterimproper disposal of sewage.

The key to provide microbiologically safe drinking water lies in understanding the various pathways by which water gets contaminated, and determining critical points prone to contamination to minimize and restrictthe entry of contaminants in drinking water source (Trevett at al., 2004). The pathway along which these contaminants move to surface waters and ground water to become incorporated in drinking water supplies is through anthropogenic activities (Ritter et al., 2002). Open defecation practices in villages near water sources,also cause direct contamination of the water bodies and result in outbreaks related to diarrhea (Sarkar et al., 2007).Fecal contamination of water is prominent in rural areas where there is lack of sanitation and hygiene, proximity to animals and their feces also add to the chances of getting infected (Luksamijarukul et al., 1994,Licence et al., 2001,Howe et al., 2002;).

Drinking water has a great potential of transporting microbial pathogens to great number of people, causing health issues, is well documented fact in all countries with every level of

economic developments. Urban communities in absence of inadequate and scarce availability of pure drinking water may be compelled to consume water of doubtful quality falling prey to such health hazards.Hence the planning and design of water supply projects should address not only the technical features but the public health aspect too. The planned strategy shall be taken into account the identification of source of supply, collection, transmission, distribution and other related aspects.

Sanitation and health protection system go hand in hand. Unavailability of safe drinking water is not only problem of developing countries but alsoof developed countries in Europe while the extent of problem is varied. Low standards of living and poor design of sewage system in the developing areas may causewater contamination (Palamuleni, 2002). Similar studies in India indicated that problems of the environment and hygiene were always varied according to the living standards and economy class of population(Nath, 2003).

1.2.3 Waterborne diseases in India

Integrated Disease Surveillance Programme(IDSP) was reported a total of 1935 outbreaks in 2015-16. The state of Maharashtra (10%), Karnataka (9%), Madhya Pradesh (7.7%), West Bengal (7.5%) and Tamil Nadu (6.3%) reported 40.5% of all outbreaks. Acute diarrheal disease and Food poisoning were found to be major causes of outbreaks reported over a period of three years consecutively.The distribution of waterborne disease outbreaks to the various states of India is shown in the figure 1.1.

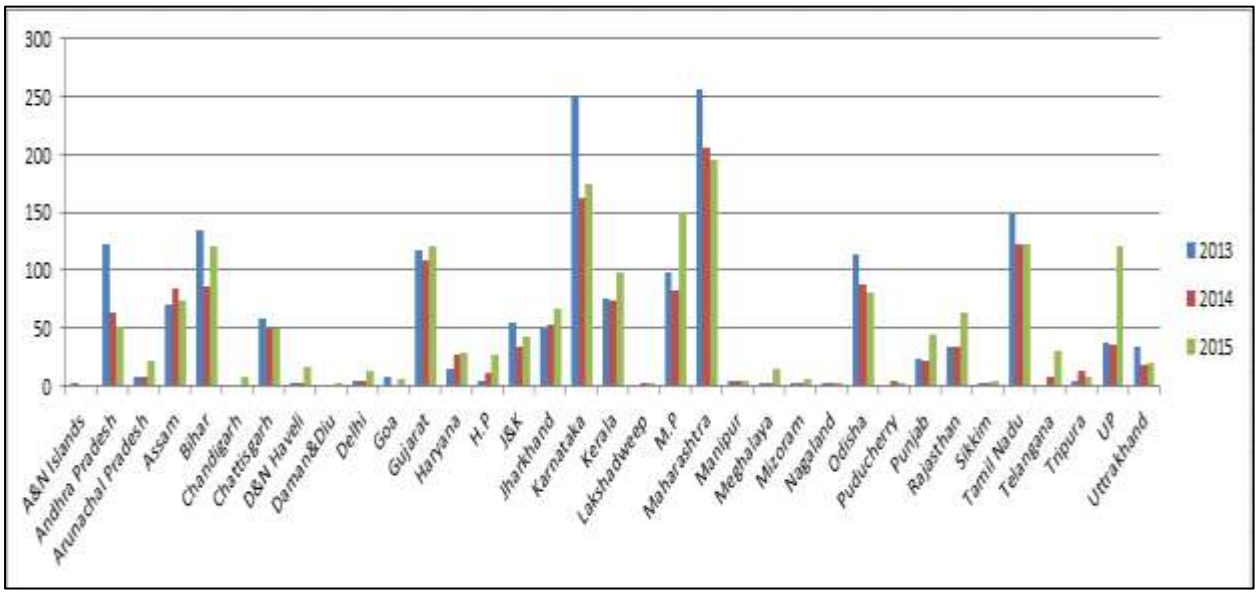


Fig1.1 State-wise distribution of waterborne disease in India. (Source: NCDC)

It is studied that approximately 72.7 percent of rural population in India does not use any water disinfection and 74 percent have no access to toilets (Mari et al., 2007). It is seen that microbial hazards have become one of the primary concerns in both developing and developed countries, thus there is a need of securing microbial safety. Microbial risks which are associated with ingestion of water contaminated with human or animal (including bird) feces are one of the major concern.

Water gets contaminated either at the source or during distribution process if the water pipes are poorly laid or maintained, or at consumer end if it is not stored properly. The risk of water contamination is higher in conditions like inadequate availability of water leading to storage of water for a long period of time can be breeding place for mosquitoes and other microorganisms, poor quality of water at source due to leaching process from agricultural or commercial land , ill-maintained water pipelines and sewer lines, improper disposal of human, animal and household wastes, and lack of sanitation (Planning Commission, 2002).

Thus, accessibility to safe and pathogen free water is a basic needas per heath perspective which can be achieved by using advanced water treatment and disinfection techniques along with adoption of personal hygiene and sanitation of surroundings.

1.3Study Area

Shimla is a beautiful hill station and state capital of Himachal Pradesh.The city sprawls over spurs of central Himalayas south of river Satluj. The average height of the city is 2130.0 m above MSL. At present Municipal Corporation of Shimla consist of 25 wards having population of about 426080 (Source: DPR, I&PH Shimla). The major land uses are located on the southern face of Shimla due to gradual slope and sunny side.

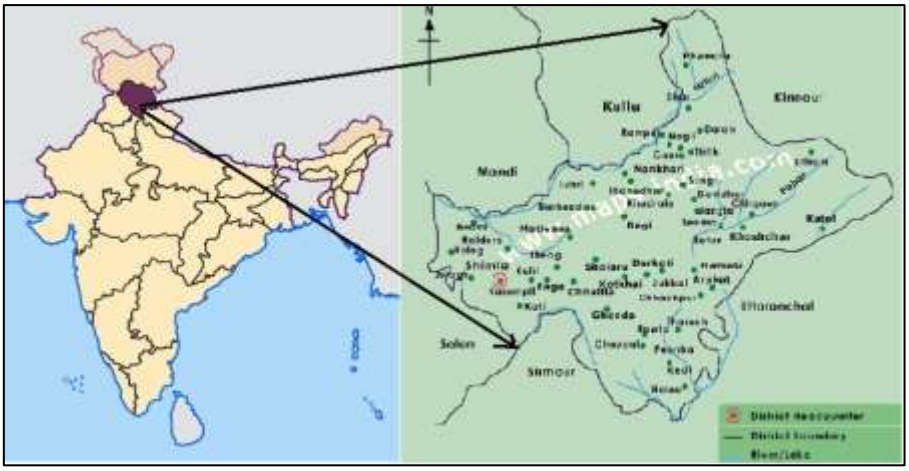


Fig. 1.2 Location of Shimla, Himachal Pradesh.

1.4Objectives

The major objectives of the project study are:

(i) Determination of prevalence of waterborne diseasein Shimla City andpopulation at risk.

(ii)Assessment of physical water quality variables and bacteriological water quality of drinking water sources, Water Treatment Plant and Distribution Systems.

(iii) Determination of degree of contamination at the various stages of water supply.

(iv) Identification of Pathogens in Drinking Water Supplies.

1.5 Organization of report

The report is comprised of five chapters.

Chapter 1provides a brief introduction about outline of this project.

In Chapter 2 different case studies were studied along with their objectives and methodology followed.

Chapter 3 deals with the research methodology providing the framework of the work to be done and procedures followed during this research.

Chapter 4 discusses the results obtained from data collection and laboratory tests experiments performed.

Chapter 5 provides the conclusion of whole project.

CHAPTER: 02
REVIEW OF LITERATURE

2.1 General

This chapter deals with the study of previous researches and case studies on similar research topics. It also includes the research methodologies and procedures adopted by them in order to achieve their objectives.

2.2 Case Studies

2.2.1 Epidemic Investigation of the Jaundice Outbreak in Girdharnagar, Ahmedabad, Gujarat, India, 2008

Chauhan et al. studied the jaundice outbreak in Girdharnagar, when jaundice cases were reported in June 2008. Investigation was started with data collection through a door-to-door survey and hospital records. The source of contamination in water supply was determined by carrying out environment investigation. Total 233 cases of hepatitis were registered. Hepatitis E IgM antibody was found in sixteen case patients out of seventeen and thus, it was concluded that the outbreak was due to hepatitis E virus. Environmental investigation confirmed drinking water was contaminated by sewage in the distribution system due to leakages aggravated by overflowing drains. It was recommended that alternative of water supply should be used if complaints regarding dirty water are made in future; which can also aid in repair of the leakages, and water quality surveillance should be done at regular intervals for prevention of such outbreak in future.

2.2.2 Investigating an outbreak of Hepatitis A in village Sharair in Himachal Pradesh. 2007

Bharti, O.K., Ramchandran V (2015) studied the water quality parameters of a contaminated water source "bawri", that lead to an outbreak of Hepatitis A in Sharair village of Himachal Pradesh. Various tests on blood and water samples were conducted. Blood samples were tested for Hepatitis A, E, B and C antigens, while water samples were tested for coliforms. Blood samples contained IgM antibodies for Hepatitis A and bawri water had high coliform count while tap water was found uncontaminated. Villagers were advised to cover the Bawri to minimize the contamination. Villages were educated about the use of chlorine tablets in their tanks to disinfect water.

2.2.3 Investigation of a hepatitis A outbreak from Shimla Himachal Pradesh. 2007

Chobe, L.P., Arankalle, V.A. (2009) investigated an outbreak of viral hepatitis at Shimla in the year 2007. It was found that maximum cases were reported from the areas getting water supply from Ashwani Khud water supply system. Samples of blood water and sewage were collected and tested for HAV-RNA by nested RT-PCR. The source of the water supply system contained treated sewage added to it at 4km upstream since a year. HAV were present in blood serum, sewage and water samples.

2.2.4 Virus occurrence in municipal groundwater sources in Quebec, Canada, 2006

Locas et al. (2007) studied groundwater quality as a source of drinking water and twelve municipal wells were monthly sampled for a one year period. Bacterial indicators, viral indicators human enteric viruses were analyzed for different sampling sites. As coliform bacteria were always present at the same time as human enteric viruses but no fecal pollution indicators were present, thus total coliforms were proved to be the best indicator of microbial degradation.

2.2.5 A hepatitis E outbreak caused by a temporary interruption in a municipal water treatment system, Baripada, Orissa.2004

Swain et al. (2009) A group of cases of acute hepatitis was reported in Baripada in the month of January 2004 and till March, 538 cases were reported. There was sudden rise in cases in February 2004 which decreased rapidly, suggesting a common source outbreak. Neighborhoods supplied by a common water supply were most affected. Ninety-one percent

of the cases reported were from zones having one common source. The water was pumped directly from a river which had not been treated during a 10-day period of a strike at the treatment plant in January and due to this interruption of the water treatment procedure, led to the supply of untreated water to the whole city.

2.2.6 Drinking water quality and source reliability in rural Ashanti region, Ghana

Arnold et al. performed various surveys and bacteriological testing of water sources in Ghana. Tests for determination of total coliforms and indicator organism i.e. E coli were performed at about eighty water sources. Various sources that were studied included rivers, shallow well, standpipes and dug wells. Residents didn't prefer the piped supply as the supply was intermittent and of poor quality. Boreholes were found to be a better source of water than standpipes against the expectations of the residents as E coli wasn't detected in them.

2.2.7 Faecal contamination of a municipal drinking water distribution system in association with Campylobacter jejuni infections, 2004

Tarja et al. studied contamination of drinking water in a rural municipality, eastern Finland in August 2004. Contamination of municipal drinking water caused due to leaching of fecal material due to rainfall due to a maintenance failure of the roof of the water storage tower, leading to a massive faecal contamination of drinking water storage and distribution system. Initially disinfection wasn't done and ground water was directly supplied, but when faecal contamination was detected and chlorination was done right after. Parameters of baiological water quality like detection of Escherichia coli, coliform bacteria and heterotrophic bacteria counts were analysed and testing for thermophilic campylobacters, Salmonella, noroviruses, coliform bacteria, E. coli, intestinal enterococci and heterotrophic bacteria were performed. Notices regarding using boiled water and disinfection practices were issued. Sampling on regular basis to monitor water quality, cleaning and maintenance was suggested to prevent such outbreaks in future.

2.2.8 A Large Outbreak of Hepatitis E among a Displaced Population in Darfur, Sudan, 2004: The Role of Water Treatment Methods, 2004

Guthmann et al. studied the importance of water treatment methods in controlling hepatitis E cases during a conflict in Darfur. For the confirmation of the outbreak, clinical and demographic data of the cases was recorded. Stool and blood samples of infected people and animals were collected and checked for IgM antibodies of hepatitis E. Along with tests on

blood samples; bacteriological analysis of water samples was also done. It was studied that, 2621 cases of hepatitis E were reported in 6 months. HEV RNA was identified in serum samples but bacteria were not identified from any sample of chlorinated water tested. This studied concluded that proper chlorination if adopted, such outbreak could have been prevented.

2.3 Other studies

2.3.1 Enumeration of Coliform bacteria in drinking water of Mughalpura, Lahore, 2011

Sulehria et al studied the drinking water at various stages in Mughalpura to check the quality of water supplied to the residents. The study included determination of fecal coliform by Multiple Tube Fermentation Technique, determination of bacterial counts and study of physic-chemical parameters like residual chlorine and pH. Thirty samples from four different stages of water supply were taken and all samples revealed the presence of coliform bacteria. The counts were greater than the counts as prescribed in WHO standards. The bacterial counts were found in greater concentration in consumer taps, followed by distribution line and main reservoir. The pH was found within the limits but, there was no residual chlorine in any of the samples. Thus, it was concluded the drinking water quality of Mughalpura is unfit for drinking purpose.

2.3.2 Biofilms in drinking water and their role as reservoir for pathogens

Wingender, J., Flemming, H.C studied the microorganisms living as clustered aggregated also known as biofilm and are the active agents in the process of biofiltration .They also cause biofouling, on the surface of technical systems. In recent studies, it was found that biofilms in drinking water distribution networks can become long-term habitats for such microorganisms. Such microorganisms include faecal indicator bacteria like E coli), Campylobacter spp., other bacterias from environment like Pseudomonas aeruginosa, and enteric viruses like noroviruses, roatviruses and adenoviruses. These organisms can get combined with existing biofilms, where they form clusters and may survive for longer period of time ranging from few days to weeks depending on environmental conditions of the place and type of organisms. Therefore, biofilms formed in drinking water systems can be taken as potential source of contamination and as a reservoir of various types of pathogenic and non-pathogenic bacteria.

CHAPTER: 03

METHODOLOGY

3.1 General

This chapter deals with the methodology followed during the research work, followed by the details of the various tests performed. The water samples were taken from the Ashwani khud WTP and the households served by it. The water samples were tested in Jaypee University of Information and Technology. The methodology followed in this research in order to achieve the objectives, is shown in the Fig. 3.

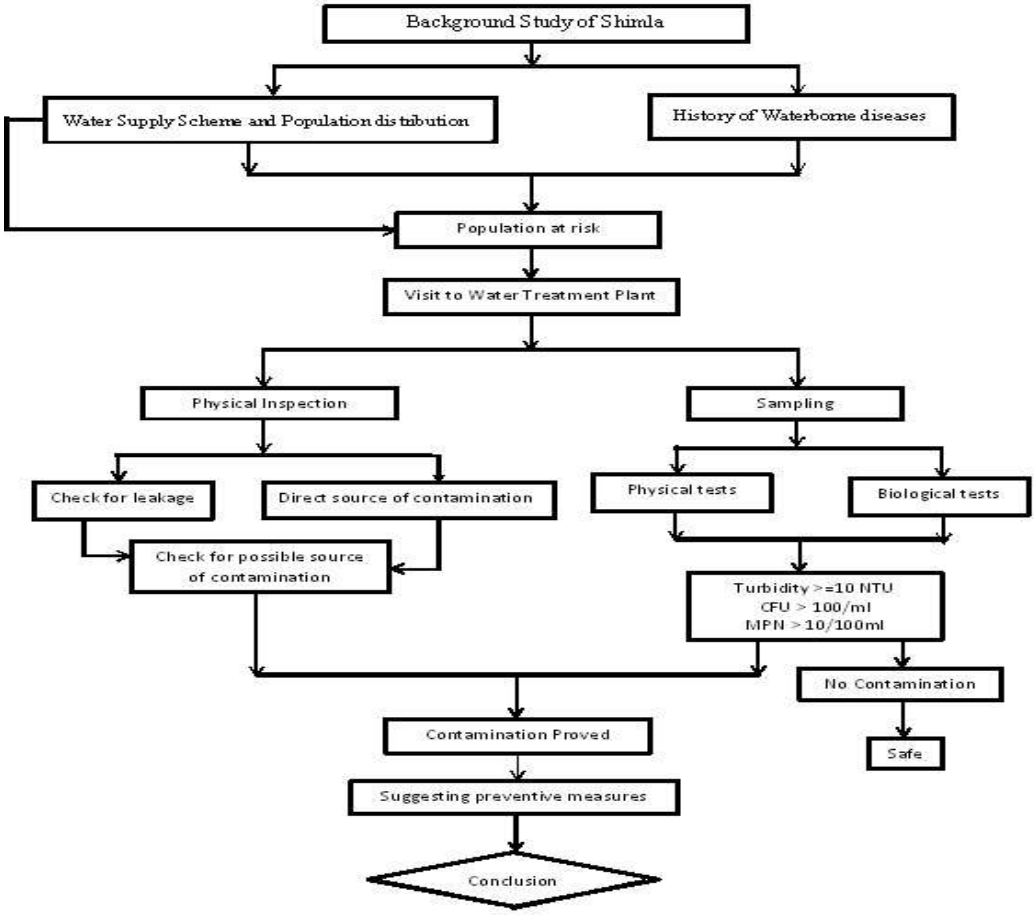


Fig. 3.1 Methodology followed during research

3.2 Background study of Shimla

Background study of Shimla was done by collecting data regarding detailed water supply scheme and history of waterborne disease outbreaks in Shimla City.

Data regarding waterborne disease was collected from Deen Dayal Upadhayay Hospital, Shimla. Diseases focused in this study are the ones that are most frequent in Shimla, and outbreaks were reported. Week-wise and area wise reporting of cases were asked (if any).The disease pattern and details about cases were studied and areas from which maximum cases were reported were noted. Data regarding detailed water supply scheme was collected and doubts were discussed with I&PH department officials.

3.3 Water sampling

According to WHO, one of the objectives of surveillance is to assess the water quality at the point of supply and at the point of use. Thus, samples must be taken from the locations that are representative of whole water source/ storage/distribution system.

3.3.1 Method and procedure

Autoclaved Plastic bottles of 500 ml capacity and 50 ml tarson tubes with stopper were used for collecting samples. Each container was washed with labolene solution, then rinsed three times with distilled water and autoclaved. The bottles were then preserved in a zipped plastic bag. The bottles used for taking samples after chlorination was done, contained 0.1 N Sodium thiosulphate to inactivate the residual chlorine which may lower the bacterial counts with action of residual chlorine with time.

Samples were taken from the source (reservoir) facing the inlet and from the underground tanks with the help of clean glass bottles, the water was then transferred to the autoclaved sampling bottles. While taking samples from the tap water, the tap outlet was flamed for few minutes to sterilize. Before collecting the sample, water was allowed to run for half a minute. The bottles were filled up to the rim, and then caps were replaced to prevent any leakage. Each container was neatly labeled with the name and date of sampling.

3.3.2 Sampling points

The samples were collected from the sampling point as marked in fig.1 and are as follows:

1. Source	4. Service Reservoir
2. Before disinfection (After filteration)	5. Tap water (Consumer end)
3. After disinfection (Clear water tank)	

Fig 3.2 Various sampling points at different stages of water supply from Ashwani Khud WTP

3.4. Physico-chemical water quality parameters

Following parameters of the water samples from the listed sampling points were studied at the time of sampling as they tend to change during storage and transportation of the samples.

1. pH
2. Turbidity
3. Temperature
4. Free residual chlorine.

The results of the analyzed parameters of water of the different locations are then compared with the related standards for drinking water prescribed by IS: 10500. The drinking water standard is given in the Table.3.1.

Table.3.1 Various parameters and their acceptable and permissible limit as per IS: 10500

Sr. No.	Parameters	Acceptable Limit	Permissible Limit
PHYSICAL PARAMETER(S)			
01	pH	6.5-8.5	No Relaxation
02	Turbidity, NTU	1	5
03	Temperature	No guideline value set	
CHEMICAL PARAMETER			

04	Free residual chlorine	0.2	1

3.4.1 Temperature.

Temperature was measured with the help of thermometer immersed directly in the water body, till it showed a constant reading. It is very important parameter from microbiological point of view, as explained before in review of literature, activity of microorganisms depends on it.

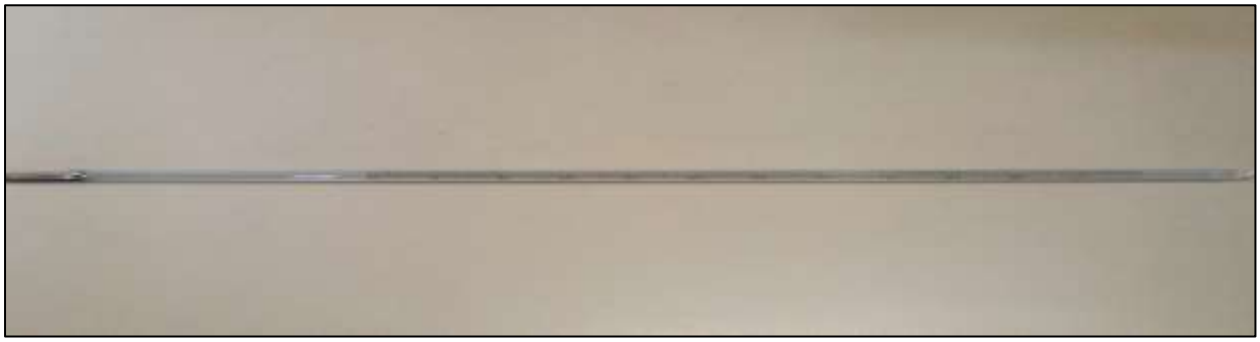


Fig. 3.3 Thermometer for measuring water temperature

3.4.2 pH

pH value is the indicator of logarithm of reciprocal of hydrogen ion activity (moles per liter) in water. Thus, it denotes the acidity or basicity of the sample. Dissolved gases such as carbon dioxide, hydrogen sulphide and ammonia also affect pH value of water. The overall pH value range for the natural water is generally between 6 and 8. The pH value obtained in the laboratory may differ as that of water at the time of collection of samples due to absorption of gases, reactions with the sediments, hydrolysis and oxidation/reduction taking place within bottle containing sample(s). Thus, the pH value must be determined at the time of collection of sample.

The pH value was determined with the help of analysis kit, shown in Figure.3.4.

Instrument was standardized with a buffer solution of pH near that of the sample and check electrode against at least one additional buffer of different pH value. Temperature was adjusted. The electrodes were rinsed and gently wiped with solution and then immersed in the sample beaker and gently stirred at a constant rate to provide homogeneity and suspension of solids. The pH value was then recorded once the instrument showed a constant value.

Fig. 3.4 Analysis kit used for determination of pH and Turbidity.

3.4.3 Turbidity

It is based on the intensity of light scattered by the water sample under standard conditions compared to intensity of light scattered by a standard suspension solution under the same conditions.

Generally, turbidimeter works on the principle of measuring interference caused by water samples due to passage of light rays. The turbidity was recorded with the help of analysis kit shown in the Figure 3.4.

The instrument was calibrated with a standard solution of 100 NTU. The sample is shaken to put solids in suspension. Wait until air bubbles disappeared. Sample is then poured into turbidimeter tube and turbidity is read directly from the instrument scale and recorded.

3.4.4 Residual Chlorine

Some amount of chlorine is still left after the disinfection process is complete, known as residual chlorine. Chlorine is unstable in aqueous solution. Any sort of agitation, exposure to air and sunlight will accelerate reduction of chlorine. So, it is recommended that chlorine determinations must be started immediately after sampling and exposure to light and agitation should be avoided.

Residual chlorine was calculated at the site with the help of ortho-tolidine reagent using water testing kit. This method is sensitive to low residual chlorine concentrations, in the range of 0 to 5 mg/l.

A test tube was washed and filled with water sample. 2-3 drops of reagent were added and shaken to dissolve. The color then obtained was noted and compared to the chart on the testing kit, shown in Figure.3.5.

Fig. 3.5 Residual chlorine color chart

3.5 Microbiological water quality parameters

3.5.1. Colony forming Units of Water Sample.

Colony forming unit is an indicator of the number of viable microorganisms in a water sample. It is defined as the number of microorganisms forming colonies, when is cultured using spread plate or pour plate method.(Prescot et al.)

The method adopted in this research was spread-plate technique. In this method, a diluted sample of bacteria or other microorganisms was dispersed over a solid agar surface i.e. of Luria agar and incubated for 24 hours at 37°C. Each microorganism or group of microorganisms forms a distinct colony.

The actual number of viable microorganisms in the water sample was calculated using the number of colonies formed and the sample dilution, using the formula:

$$\text{CFU/ml} = \frac{\text{No. of colonies x Dilution factor}}{\text{Total volume of sample spread}} \quad \text{.....(1)}$$

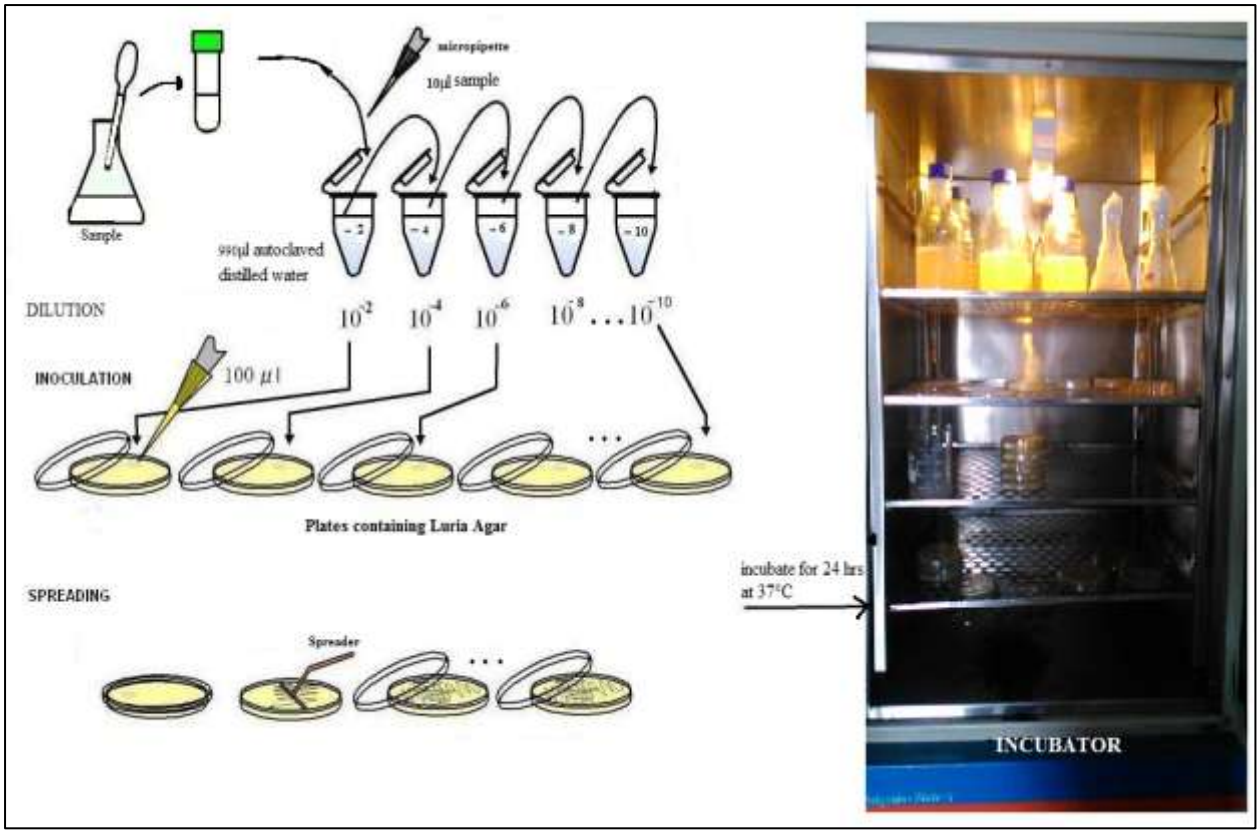


Fig. 3.6 Process flow diagram for determination of Colony forming units

3.5.2. Enrichment of Water Sample with Media.

As the number of pathogenic microorganisms is typically low in drinking water systems and sources, their recovery is low as they're in stressed conditions. Thus, chances of detecting pathogenic bacteria will be greater by using enrichment before selective plating. This allows organisms to grow before selective pressures are applied. Subsequent inoculation into enrichment media enhances the growth of microorganisms, which can further be detected by streaking on solid selective media.

The enrichment media selected in this research is Luria Broth, prepared by suspending 2 grams in 100 ml distilled water, then dispensed in tubes. It is then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and then allowed to cool. Then tubes are marked as per the sample name along with the date of experiment. The tubes are then inoculated with the water samples. The inoculated tubes are then incubated for 24 hours at 37°C. The result of growth, denoted by change in color and turbidity of the media is then noted.

3.5.3. Isolation of different bacteria from Water Sample.

Isolation of the bacteria is done with the help of selective media, which enhances the growth of one type of bacteria inhibiting the growth of other bacteria grown in the process of enrichment.

In this step, the loop is sterilized by putting the loop in flame till it turns red hot and is then allowed to cool on the wooden stand. The growth from the bacteria enriched tube is picked with the help of sterilized loop and it is then streaked on the solid selective media plate as shown in the Figure. 3.7.

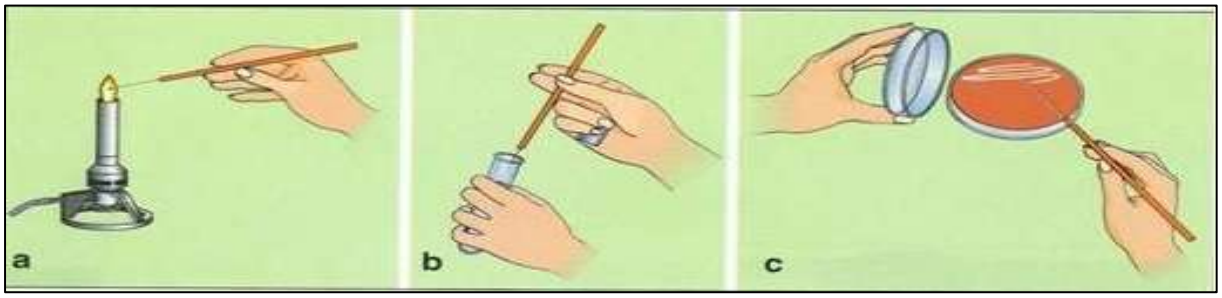


Fig.3.7 Diagram depicting streaking process.

The following selective media were selected as per the frequent outbreaks and the responsible bacteria:

3.5.3.1 Mac Conkey Agar

Mac Conkey agar is used for the isolation and differentiation of gram-negative enteric bacilli. Thus, it helps in identification of fecal contamination. Mac Conkey agar is selective and differential medium, inhibiting Gram-positive bacteria and allowing only Gram-negative to grow. During the lactose fermentation, pH drops around the colony formed causing change in color. Mac conkey agar plate is shown in the Figure.3.8.

This agar differentiates between gram-negative bacteria that can ferment lactose from those which cannot ferment lactose.

1. Lactose Positive

By utilizing the lactose available in the medium, Lactose fermenting bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* will produce acid, lowering the pH of the medium below 6.8, resulting in the appearance of pink colonies. The bile salts precipitate in periphery of the colony, causing the medium around the colony to become hazy.

2. Lactose Negative

Non-Lactose fermenting bacteria such as *Salmonella*, *Proteus* species, *Yersinia*, *Pseudomonas aeruginosa* and *Shigella* cannot

utilize lactose, utilizing peptone, further forming ammonia, which raises the pH.Thus, leads to the formation of white/colorless colonies on the agar.

3. Slow

Few organisms ferment lactose slowly or weakly. The example includes Serratia and Citrobacter.

4. Mucoid colonies

Few organisms like *Klebsiella* and *Enterobacter*, produce mucoid colonies that are moist and sticky in appearance. This happens because of organism that is producing a capsule made from the lactose sugar in the agar.

Fig. 3.8 Non-streaked and labeled plates of Mac Conkey, XLD and TCBS agar

3.5.3.2 Xylose-Lysine Deoxycholate Agar

Xylose-Lysine Deoxycholate Agar also known as XLD agar is a selective growth medium used for the isolation of Salmonella and Shigella species. The non-streaked plate of XLD agar is shown in the Figure.3.8.

The result can be analyzed as per the growth characteristics, described below:

1. *Salmonella* species: formation of red colonies, few with black centers. The agar itself will turn red due to presence of Salmonella-type colonies.

2. *Shigella* species: formation of red colonies.

3. Coliforms: formation of yellow to orange colonies.

4. *Pseudomonas aeruginosa*: formation of pink, flat, rough colonies. Such type of colony can be easily mistaken for Salmonella due to color similarities.

3.5.3.3 Thiosulfate-citrate-bile salts-sucrose Agar

Thiosulfate-citrate-bile salts-sucrose agar, also known as TCBS agar, is a type of selective agar that is used to isolate *Vibrio* spp. It is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* as well as the other vibrios species. TCBS agar inhibits the growth of Enterobacteriaceae, gram-positive bacteria. The alkaline pH of the TCBS medium enhances recovery of *V. cholerae* and inhibits the growth of other bacteria.

The non-streaked plate of TCBS agar is shown in Figure.3.8. The results can be interpreted on the basis of following growth characteristics:

1. *V. cholerae*: formation of large yellow colonies.
2. *V. parahaemolyticus*: formation of colonies with blue to green centers.
3. *V. alginolyticus*: formation of large yellow mucoidal colonies.
4. Proteus/Enterococci: Partial inhibition. If growth, colonies are small and yellow to translucent.
5. Pseudomonas/Aeromonas: Partial inhibition. If growth, colonies are blue.

3.5.4 Most Probable Number of Water Sample.

Along with isolation of bacteria on the selective media, determination of fecal contamination in the water sample was done with the help of Multiple tube fermentation method.

3.5.4.1 Multiple Tube Fermentation Method

The Multiple-Tube Technique is a three stage procedure that consists of a presumptive test, confirmed and completed test.

Presumptive test: Mac conkey broth consisting of inverted Durham tube was inoculated with the water sample to determine if acid and gas fermentation of lactose is occurring, indicating the presence of coliforms. Three different volumes of sample water were inoculated in replication. The result is checked and noted at every 24 and 48 hours of incubation. If gas and acid was produced in any of the tubes, coliforms were presumed to be present in the water sample.

Orange coloration indicates the presence of lactose fermenting bacteria due to the decrease of pH caused due to acid production. Yellow coloration indicates the presence of non-lactose

fermenting bacteria. The tubes showing gas and acid production were noted. The result is expressed in the terms of Most Probable Number (MPN) denoted as MPN /100 ml of sample. The MPN value is calculated using the table standardized by WHO shown in Table 3.2

Confirmed test: Eosin-Methylene Blue agar is used to identify positive lactose fermentating tubes as fecal coliforms. The tube showing positive presumptive test was then streaked onto EMB agar.

Complete test: Representative colonies were chosen that showed positive result on EMB agar, inoculated in Lactose broth and incubated for 37°C for 24hours. If gas is production show the confirmation of coliforms and complete the test.

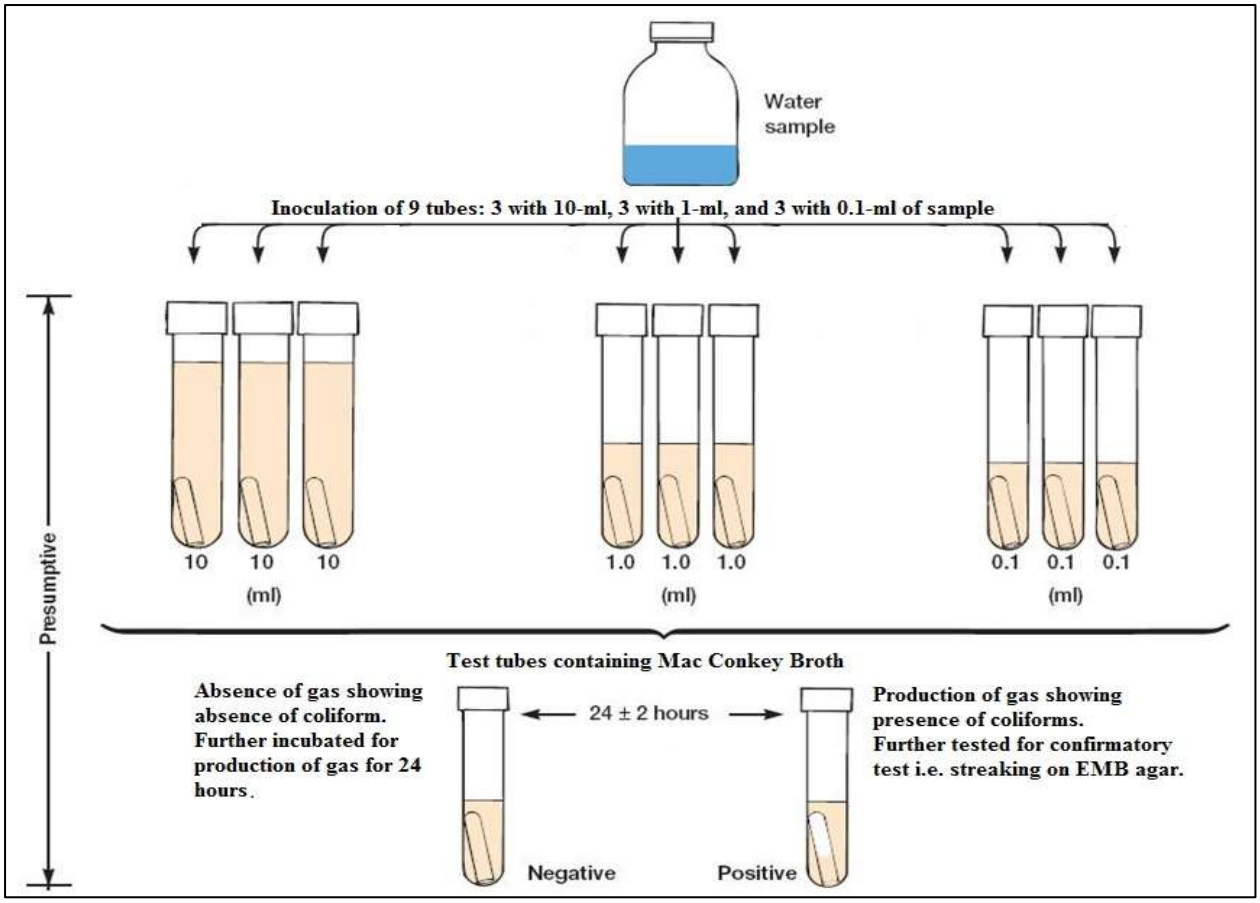


Fig. 3.9 Diagram showing Multiple tube fermentation process.

Table: 3.2 MPN values per 100ml of sample with 95% confidence limits when three 10-ml, three 1-ml, and three 0.1-ml inoculations are used

No. of tubes giving a positive result			MPN (per 100 ml)	95% confidence limits	
3 of 10 ml	3 of 1 ml	3 of 0.1 ml		Lower	Upper

0	0	1	3	<1	9
0	1	0	3	<1	13
1	0	0	4	<1	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	49
2	2	0	21	4	47
2	2	1	28	10	149
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	379
3	1	0	48	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470

3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

3.5.5. Biochemical tests for the identification of bacteria

After determination of colony forming units that showed the presence of bacteria, isolation of bacteria to check the various types of bacteria present in the water sample, along with determination of coliforms to check the possibility of fecal contamination, biochemical test was performed to determine the species of bacteria present in the water sample(s) using IMVic test.

IMViC Test

In IMViC test consist of four biochemical tests, carried out individually. IMViC test is carried out for the identification of the members of Enterobacteriaceae family (Paniker,C.K.J.,2005).

IMViC test stands for individual test with every letter denote:

1. I – Indole production test.
2. M – Methyl red test.
3. V- Voges- Proskaeur test.
4. C – Citrate utilization.

3.5.5.1 Indole production test:

This test is performed in a peptone water culture after incubation of 24 hours at 37°C. The test demonstrates the production of indole from tryptophane. Two-three drops of Kovac's reagent is added and shook gently. Formation of red ring denotes a positive reaction.

3.5.5.2 Methyl red test (MR):

This test is used to detect the production of acid during fermentation of glucose. Few drops of 0.04% solution of methyl red is added to culture in glucose phosphate medium, that had been incubated for 24 hours at 37°C, mixed properly and read at once. Red color denotes a positive while production of yellow color signifies a negative test.

3.5.5.3 Voges-Proskauer test (VP):

In Voges-Proskauer test, butanediol fermentation is determined by detecting acetoin. The culture in glucose phosphate medium that had been incubated for 24 hours at 37°C is taken and 0.6 ml of alpha naphthol and 0.2 ml of KOH solution per ml of culture broth media is added. It is further kept open to increase aeration. The result is noted after 10-15 minutes after the reagents were added.

3.5.5.4 Citrate utilization:

In this test Simmons' citrate medium slant was used. Simmons' citrate agar differentiates gram-negative bacteria on the basis of citrate utilization, thus, useful for selected organisms that use citrate as its main carbon and energy source. The Simmons's agar was prepared, sterilized by autoclaving and the slants of this media were prepared. Further this slant was streaked and punched with the culture using a sterilized loop and incubated for 24 hours at about 37°C. After incubation the slant was observed for change in color and the results were recorded.

3.5.5.5 Triple sugar iron slant test:

The Triple Sugar Iron (TSI) test is used to determine microorganism's ability to ferment sugars and to produce hydrogen sulfide. It is often used for the selective identification of enteric bacteria including *Salmonella* and *Shigella*. The TSI agar was prepared, sterilized by autoclaving and the slants of this media were prepared. Further this slant was streaked and punched with the culture using a sterilized loop and incubated for 24 hours at about 37°C. After incubation the slant was observed for change in colour and the results were recorded. The result is interpreted as:

1. Bacteria that ferment sugars to acids, causing change in color from red to a yellow. Many bacteria that can ferment sugars in the anaerobic bottom of the tube are enterobacteria.

2. Sometimes blackening of the medium is observed at the bottom of the medium, it is due to the fact of thiosulfate anion utilization, visible as a black precipitate. Sulfide-producing bacteria include *Salmonella and Citrobacter spp.*

3. A bacteria that ferments glucose, initially causes a yellow slant/yellow butt (acid/acid reaction) after 8 hours but then converts to a red slant/yellow butt after 24 hours (alkali/acid reaction). When it ferments both lactose and glucose, it results in a yellow slant/yellow butt tube.

CHAPTER: 04

RESULTS AND DISCUSSION

4.1 General

This chapter provides a descriptive summary of water supply scheme and the history of waterborne disease in Shimla City. It also discusses the water quality parameters in terms of physico-chemical and bacteriological characteristics of water samples taken from Ashwani Khud source.

4.2 Water supply scheme and waterborne disease in Shimla City

4.2.1 Water supply scheme

Shimla city water supply started as a feeder of small population of 16000 in 1875, which grew to a larger proportion further augmented and improved over the years. Despite the improvements carried out so far, scarcity of water still prevails in many parts of the city particularly in special areas added within the municipal boundary.

Depletion of the yield of sources during lean period cause widening of demand and supply gap, resulting in much hardships and miseries to the inhabitants of the city. The state administration has conceived a plan to bring water from Koldam Reservoir (Lift Water Supply Scheme from Koldam Reservoir) to tide over the water crisis.

4.2.1.1 Source of water supply Shimla

The water supply facilities were introduced in Shimla city more than 130 years ago by pumping water from nearby spring sources. The city grew over the years, increasing its boundary and its population with subsequent augmentation of water supply to meet the needs of the growing population. There are six sources of water supply with total capacity of 57.61 MLD with total six augmentation ,with the sixth augmentation in the year 2008 (Giri).

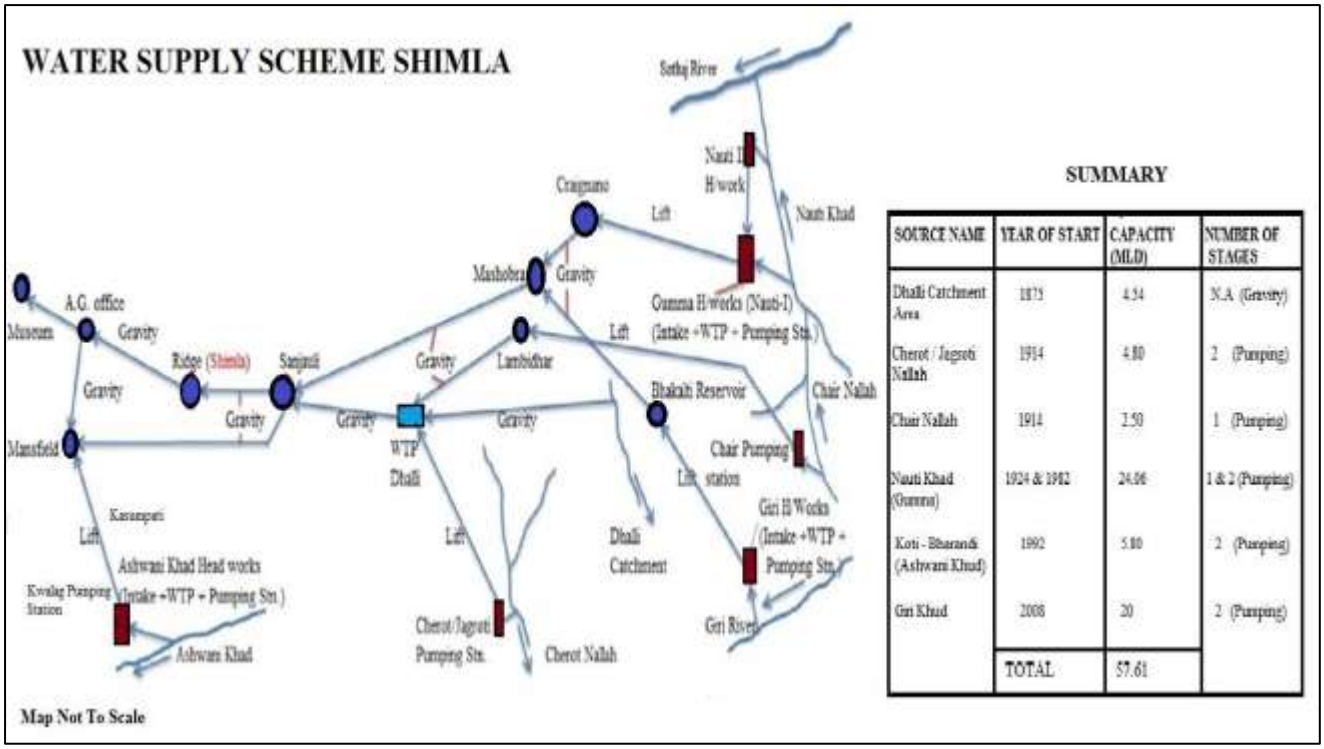
The water, which is being received from different sources for Shimla Town for distribution, is described in Table 4.1.

Table 4.1 Different sources of water and their transmission details.

Sr. No.	Source Name	Transmission Type	Year of Start	Installed Capacity MLD	Present Supply MLD	Areas/Zone Served

01	Dhalli Catchment Area	Gravity	1875	4.54	2.35	Dhalli
02	Cherot / Jagroti Nallah	Pumping	1914	4.80	3.50	Dhalli , Sanjauli
03	Chair Nallah	Pumping	1914	2.50	1.29	Kufri
04	Nauti-Khud (Gumma)	Pumping	1924 & 1982	24.06	18.10	Sanjauli, Ridge, Mansfield
05	Ashwani Khad	Pumping	1992	5.80 (10.06)	5.40	Kasumpti, New Shimla, Knollswood
06	Giri Khad	Pumping	2008	20	12.65	Tutikandi, Kamna-devi
	Total			57.61	43.29	

Initial capacity of Ashwani khud as a source was 10.06 MLD but later reduced to 5.80 MLD due to change in its source to Koti-Bharandi Nallah, in response to the major jaundice outbreak in the year 2015-2016. The general layout of water supply scheme of Shimla city is shown in Figure 4.1.



SUMMARY

SOURCE NAME	YEAR OF START	CAPACITY (MLD)	NUMBER OF STAGES
Dhalli Catchment Area	1875	4.54	N.A (Gravity)
Cherot / Jagroti Nallah	1914	4.80	2 (Pumping)
Chair Nallah	1914	2.50	1 (Pumping)
Nauti Khad (Gumma)	1924 & 1982	24.06	1 & 2 (Pumping)
Koti - Bharandi (Ashwani Khud)	1992	5.80	2 (Pumping)
Giri Khud	2008	20	2 (Pumping)
	TOTAL	57.61	

Fig.4.1 General Layout of water supply scheme Shimla

The source focused in this research is Ashwani Khud. The details of the distribution system of Ashwani Khud are discussed in detail in further sections.

4.2.1.2 Service Reservoirs

Water from the above-listed sources are treated in their respective water treatment plants and then pumped barring Dhalli catchment source, operating under gravity conditions.

Water, thus pumped or gravitated are stored in ten service reservoirs located at suitable sites covering the MC Shimla are given in the Table 4.2.

The reservoir studied in this research is Kasumpati under-gravity tank in Kasumpati, Shimla as it receives water from Ashwani Khud water treatment plant, chlorination is done and water is thus sent further in the distribution network such that the water received at the consumer end is pathogen free.

Table 4.2 Reservoirs in Shimla City: Capacity and type.

Sl. No.	Reservoirs	Capacity in ML	Type
1.	Carignano	3.01	Under-gravity
2.	Sanjaulli	8.78	Under-gravity
3.	Ridge	4.63	Under-gravity
4.	Mansfield	3.63	Under-gravity

Sl. No.	Reservoirs	Capacity in ML	Type
5.	Kasumpti	2.00	Under-gravity
6.	Kasumpti	0.227	Over-head
7.	Vice-regal lodge	0.23	Over-head
8.	Jakhu	0.32	Over-head
9.	Boileaugunj	0.24	Under-gravity
10.	Balancing Reservoir at Masobara	3.00	Under-gravity
	Total	**26.447**	

The total capacity of existing reservoirs is 26.447 ML as listed in Table 4.2. Newly developed areas of BCS, Chakkar, Totu do not have separate service reservoirs and they are fed from the existing ones leading to considerable loss in pressure at the consumer end of the network.

4.4.2 Water supply zones and water demand

Shimla City is divided into fourteen water supply zones in order to meet the water demand of the population and balancing it with the yield of limited sources. Figure 4.2 shows the various water supply zones along with municipal zones in Shimla.

Fig. 4.2 Water supply zones Shimla

It can be seen that Sanjauli and A.G. Office zones are the largest water supply zones in Shimla. Kamna Devi and Chakkar zone are shown separately along with numbered zones of Municipal Corporation. Table 4.3 provides in depth information about the fourteen delineated

water zones based on topography, areas covered, population and their respective water demand.

Table 4.3 Water supply zones: Population and water demand

Name of Zone	Population	Areas Covered	Demand MLD
1.Sanjauli	41310	Sanjaulli Bazaar, Engine Ghar, Nav Bahar, Snowdown, Jakhoo, Sangti Pumping Station,Grand Hotel,	5.1
2.Bharari	29210	Bharari, Harvington, Kuftu, Anu, Bermu	3.2
3.Ridge	40758	Telegraph office, Krishna Nagar, Sabzi Mandi, Ripon, Lalpani, Western Command, Ram Bazaar, Middle bazaar	3.65
4.High Court	35882	Lower High Court area, Paradas Garden, Kanlog, Talland	3.46
5.BCS	22420	BCS, Khalini, Forest Colony	2.62
6.A.G. Office	44425	Kaithu, Annandale, AG Office, Ram nagar, Vidhan Sabha, Chaura Maidan, Tuti Kandi, Raj Bhavan, Win Gate	4.45
7.Vice Regal lodge	40155	Institute of Advanced Studies, Tilak Nagar, Ghora Chowki, Hanuman Temple	3.54
8.Mansfield	16735	Mansfield, Marina, Secretariat, Chota Shimla, Brock Hurst, Govt. School	1.81
9.Kasumpti	40302	Kasumpti Colony, Lower Brock Hurst, Patti Rehana, Patina Mehli, Pantha Ghati, Patelog	3.56

10.University	45152	University Complex, Summer Hill, Govt. Quarters, Shiv Mandir	4.10
11.Kamna Devi	3505	Hill of Kamna Devi, Forest Colony	0.40
12.Chakkar	13164	Sandal Hill, Tara Devi, Shoghi	1.22
13.Totu	32617	Tutu Bazaar, Jutogh, Dhamida	4.16
14.Dhalli	20448	Dhingu Devi Mandir, Dhalli Bazar, Indira Nagar, Part area of Mashobra	2.40
Total	426083		43.67

The variation of water supply demand with respect to various water supply zones along with its population is shown in Figure 4.3. Kamna Devi zone have the least water demand while Sanjauli and A.G. Office are the largest zone in terms of area and water demand.

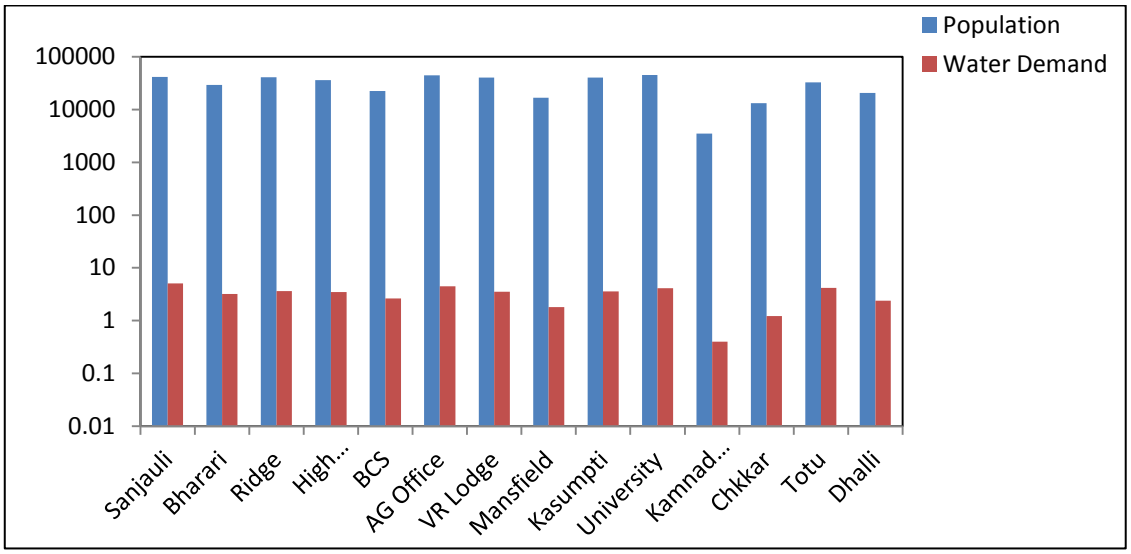


Fig. 4.3 Variation of water demand in various water supply zones of Shimla City.

4.2.3 Waterborne disease in Shimla City

Shimla is a beautiful hill station, cool climate and moderate humidity attracts many tourists from round the world so it does to the microorganisms. Pathogenicity of these microorganisms determines if they are harmful or not. Information regarding diseases that are spread by contaminated water is studied and the root cause of such disease is tried to be determined.

The data regarding the waterborne disease/outbreaks was collected from Deen Dayal Upadhyay Hospital, Shimla. The Table 4.4 shows the waterborne disease cases reported in Shimla City from the year 2008 to early 2016. The majority of cases were reported were of

Diarrhea, typhoid and Jaundice, with Jaundice being the most prevalent disease. For the first time 23 deaths were reported due to Jaundice in the year 2015-2016.

It can be seen that there is sudden rise in cases which can be due to increase in population and the supply of unsafe drinking water. Diarrhea and typhoid, found out to be more prominent disease in the Shimla city while jaundice is the most highlighted one.

Trend of jaundice cases is found to increase from 2008 till maximum cases of 1681 in the year 2015-16 which is about 300% of initial cases of jaundice in 2008-09.Cases of diarrhea were maximum in 2012-13 and 2014-15 with 3453 and 3449 cases respectively, while cases typhoid tend to increase suddenly from 2012-13 peaking at 3539 in 2013-14 which further decreased till 2015-16.

Table 4.4 Waterborne diseases in Shimla City.

YEAR	DIARRHEA	JAUNDICE	TYPHOID	DEATHS
2008-09	742	425	165	NIL
2009-10	1232	126	192	NIL
2010-11	2664	67	247	NIL
2011-12	1653	181	355	NIL
2012-13	3453	236	3775	NIL
2013-14	2926	393	3539	NIL
2014-15	3449	182	3249	NIL
2015-16	982	1681	1266	23
TOTAL	**17101**	**3291**	**12788**	**23**

The yearly distribution of waterborne disease like diarrhea, jaundice and typhoid cases are plotted and shown in the Figure 4.4. There was sudden fall in the cases during the year 2015-16, while the cases of jaundice were found maximum during all these years.

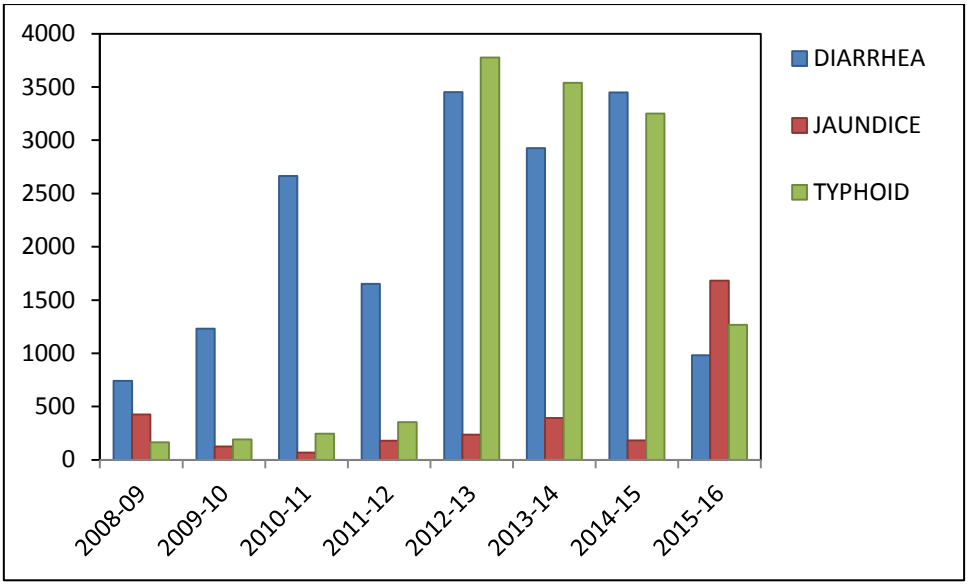


Fig. 4.4 Waterborne disease in Shimla City from 2008-2016

4.2.4 Jaundice- The most prevalent disease in Shimla

Recent jaundice outbreak in 2015-16, was highlighted to the whole nation due to about 1600 cases in 3 months and also because of first time deaths were reported because of it. According to a report of IDSP, the cause of this outbreak was hepatitis E virus which is most dangerous and can even be deadly.

Another jaundice outbreak which was most heard of were in the year 2013-14 and 2007 respectively. It was reported that cause of 2007 jaundice was hepatitis A virus (Bharti and Ramachandran) which if treated properly can minimize the illness and suffering. The data regarding jaundice is used as a base to determine critical zone and their respective population prone to such or similar outbreaks.

4.2.4.1 Jaundice Outbreak (December 2015 -March 2016)

According to local residents, jaundice outbreaks in Shimla are one of the common phenomena like snowfall. It has become the major health problem due to its increasing frequency and ability to affect a large population at the same time.

In Shimla, first case jaundice had been reported in the second week of December, which kept on rising till end January. The last case was reported in the month of March, 2016.

Jaundice is the disease of rainy season but one of its reasons in striking in the winter season may be due to global warming. The delayed snowfall or delayed winters and moderate sunshine and moisture provide favorable condition for the microorganisms to grow, which in turn if neglected during the water treatment process may pose harmful health hazard.

Following Table 4.5 show the week wise distribution of jaundice cases in Shimla City. A total of 1692 cases were received with the maximum number of cases were in 6th -7th week of the outbreak. The first case was reported in the first week of December while the last was received during second week of March.

Table 4.5 Week-wise distribution of Jaundice cases in Shimla City, 2015-16

Week	Cases	Week	Cases
Week 50	2	Week 5	141
Week 51	23	Week 6	138
Week 52	38	Week 7	85
Week 53	127	Week 8	64
Week 1	179	Week 9	48
Week 2	331	Week 10	19
Week 3	324	Week 11	8
Week 4	162	Week 12	3

Figure 4.5 shows the week wise distribution of jaundice cases in Shimla City in the year 2015-16. As seen in the graph, cases peaked in 6-7th week and decreased rapidly, indicating showing common source problem as seen in Baripada, Orissa (Swain et. al) which also had the similar pattern, peaking at 6-7th week.

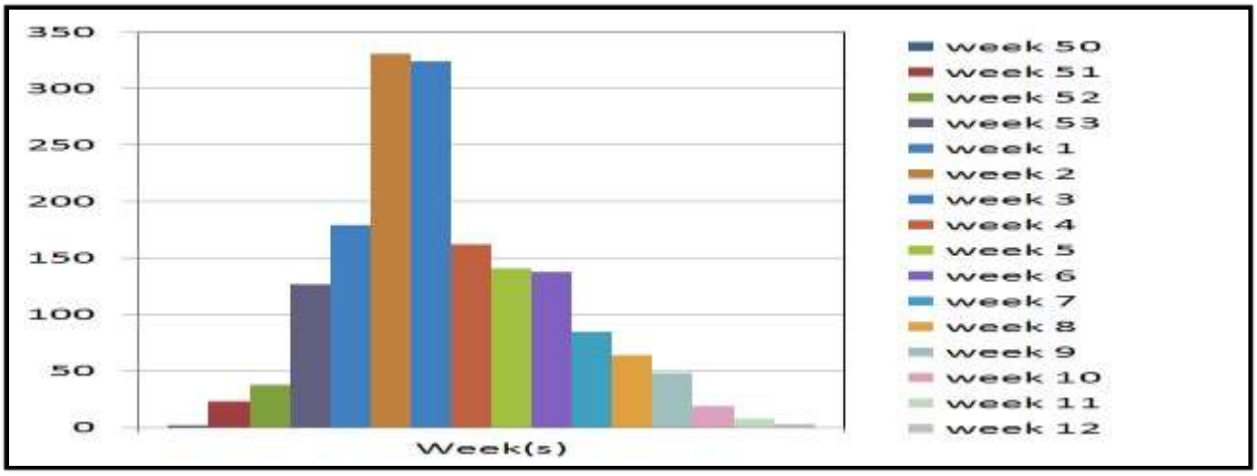


Fig. 4.5 Week-wise distribution of jaundice cases in Shimla (2015-16)

Another similarity in these two cases is both of the outbreaks were caused by hepatitis E virus and resulted due to sewage outbreak. The outbreak pattern is compared in Figure 4.6.

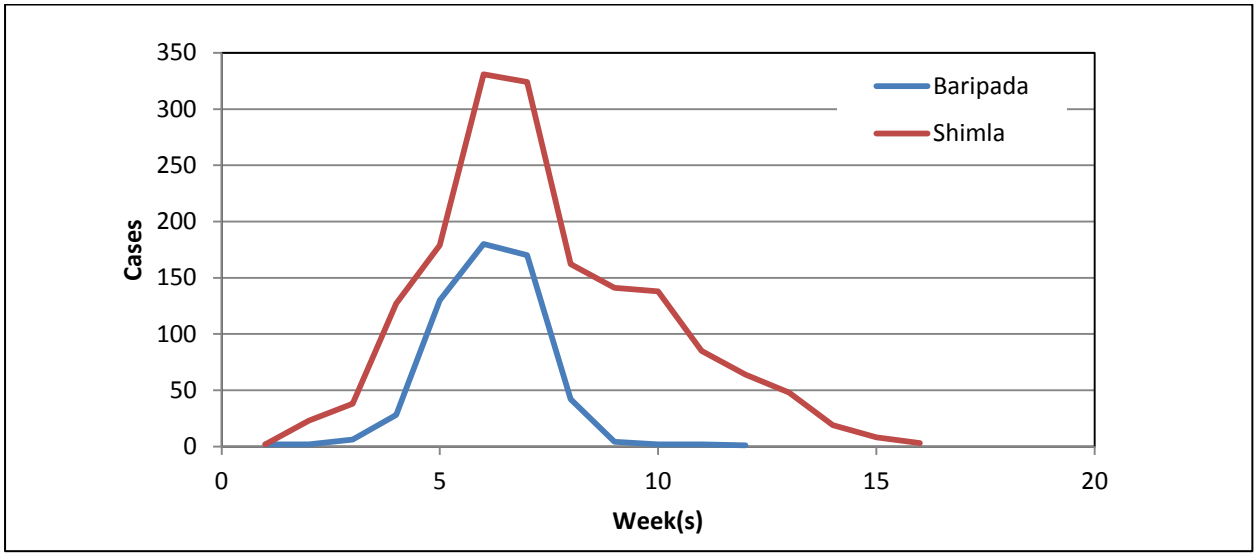


Fig. 4.6 Comparison of outbreak pattern of Shimla and Baripada.

Area wise distribution of jaundice cases

The area wise distribution of jaundice cases during 2015-2016 is shown in the Table 4.6. It can be seen that maximum number of cases were reported from Vikas Nagar, Khalini, Kasumpati, Chota Shimla and Sanjauli.

Table 4.6 Area-wise distribution of Jaundice cases.

AREA	CASES	AREA	CASES
Vikas Nagar	176	Panthaghati	89
Khalini	139	BCS Shimla	59
Kasumpti	130	Mehali	63
Chota Shimla	105	Bembaloi	27
Sanjauli	89	Dhalli	18
New Shimla	73	Others	713

As shown earlier Vikas Nagar, Kasumpati, Panthaghati, and some places of Saunjauli were served by Ashwani Khud. This lead to an inference this outbreak is result of a common source problem.Variation of reported jaundice cases with area in the year 2015-2016 is shown in Figure 4.7.

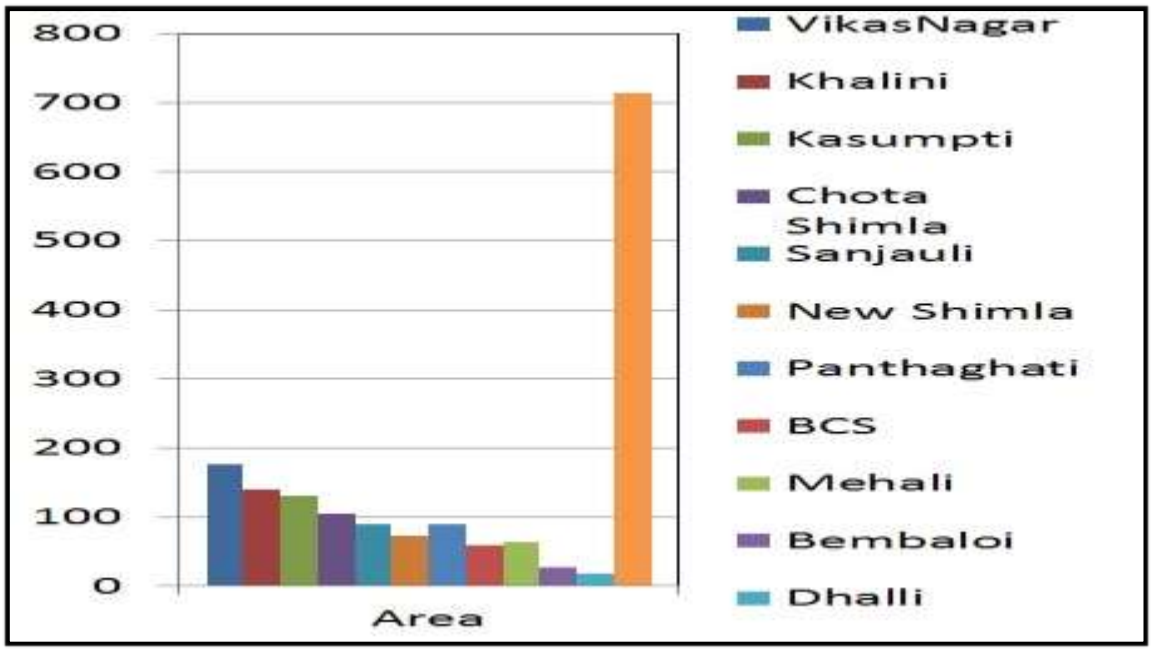


Fig 4.7 Variation of cases registered with respect to zone

4.2.4.2 Jaundice Outbreak (Jan 2007 -May 2007)

Previously, jaundice outbreak occurred at Shimla Himachal Pradesh between periods January-March 2007 (Bharti and Ramchandran).

Week-wise distribution of jaundice cases is shown in Table 4.7. The first case was reported on January 21, 2007 and approximately 450 cases were reported till February 23, 2007. This was caused because of Hepatitis A.

Table 4.7 Week-wise distribution of jaundice cases 2007

Week	Cases	Week	Cases
Week 1	93	Week 10	35
Week 3	103	Week 11	31
Week 4	119	Week 12	32
Week 5	258	Week 13	27
Week 6	240	Week 14	16
Week 7	155	Week 15	8
Week 8	97	Week 16	13
Week 9	49	Week 17	9

Week wise distribution of jaundice cases of 2007 are plotted in the Figure 4.8. As it can be seen by the graph maximum cases peaked at 5-6th weeks and then began to fall from 7th week.

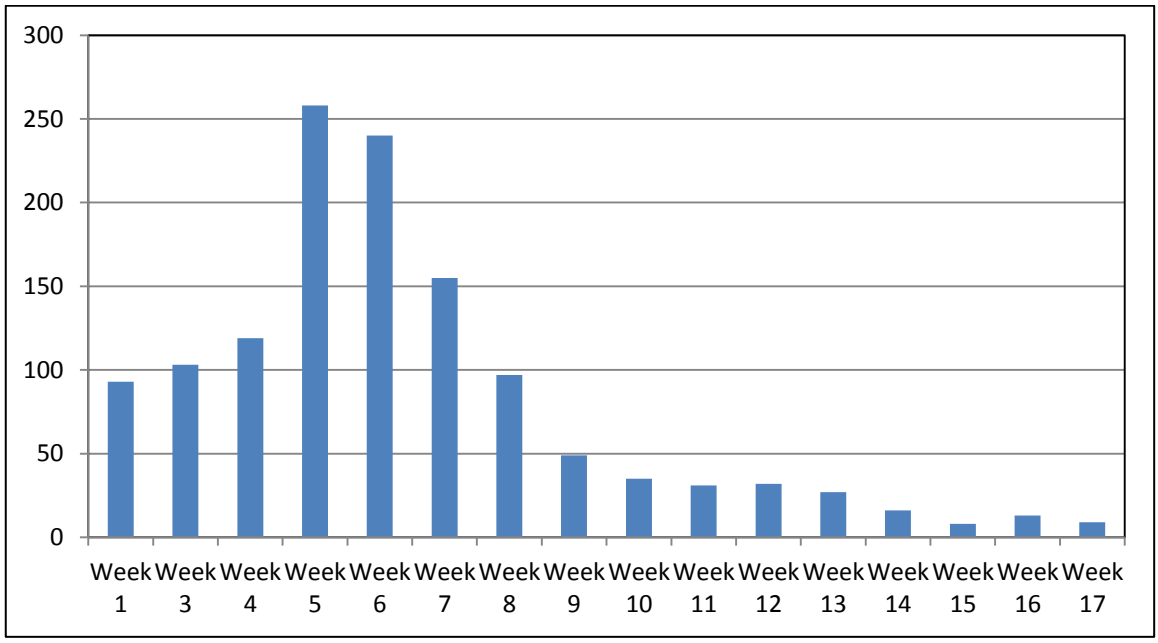


Fig. 4.8 Week-wise distribution of Jaundice cases 2007

Table 4.8 shows the distribution of jaundice cases in the various zones of Shimla City in the year 2007. Maximum cases were reported from Kasumpati area followed by Chota Shimla and Khalini.

Table 4.8 Area wise distribution of jaundice cases 2007

AREA	CASES
Kasumpti(Mehli,Jivnu Colony,DevNagar,New Shimla)	377
Chhota Shimla (Sect., Brockhurst, Flower dail)	100
Khalini (Kanlog, Talland)	92
Annadale (Kaithu, Police Line)	45
Boileauganj(Summer Hill , Chakkar, Chaura Maidan)	41
Other Areas	29

As seen in the Table 4.8 and Figure 4.9, again maximum cases of jaundice were received from Kasumpati, Chhota Shimla, Khalini, while Annadale and Boileauganj also recorded much higher number of cases compared to the 2015-16 outbreak.

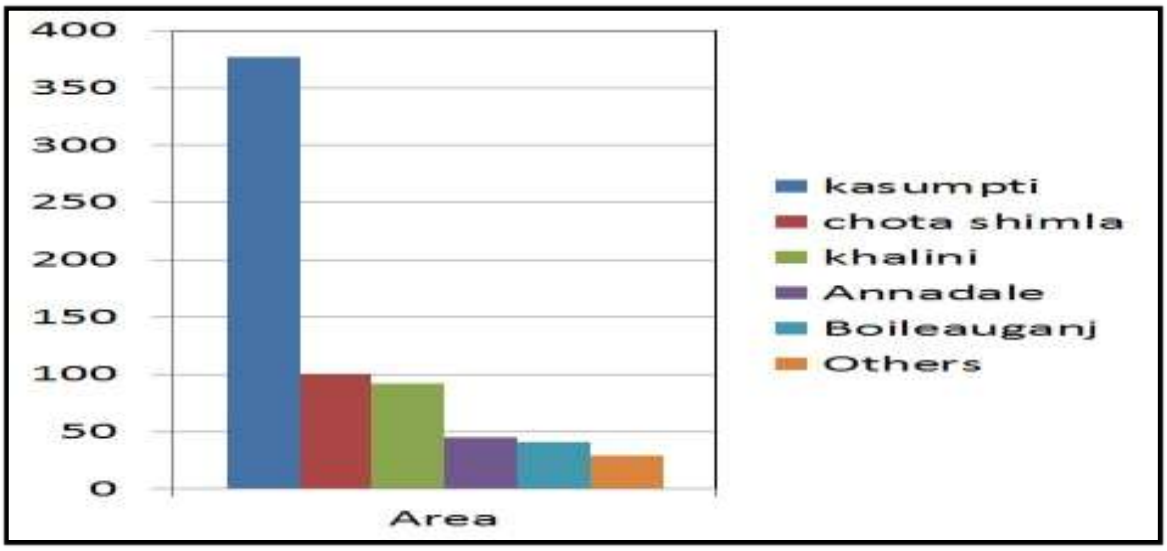


Fig 4.9 Variation of cases registered with respect to zone 2007

4.3 Conclusion

From the results shown in the Figure 4.7 and 4.9, it can be concluded that the regions of Kasumpati, Chotta Shimla, Khalini, Sanjauli are more prone to jaundice outbreaks. Further, as explained in the section 4.4.2 water zones and their respective source, Kasumpati zone and some parts of Chota Shimla and Sanjauli received water from Ashwani Khud. Thus, it lead to conclusion that Ashwani Khud was the sole source of jaundice outbreaks in Shimla during the year 2015-16 and 2007.

4.4 Ashwani Khud Water Treatment Plant

Ashwani Khud was allocated as a source for water supply by fifth augmentation in April, 1992. WTP was constructed in the year 1992, since then it is in working condition and it is being maintained time to time.

Ashwani Khud, source has six main contributing tributeries namely – Malyana Nallah, Sanan Nallah, Housing Board Nallah, Jagroti Nallah, Koti Nallah, and Bharandi Nallah and is shown in Figure 4.10.

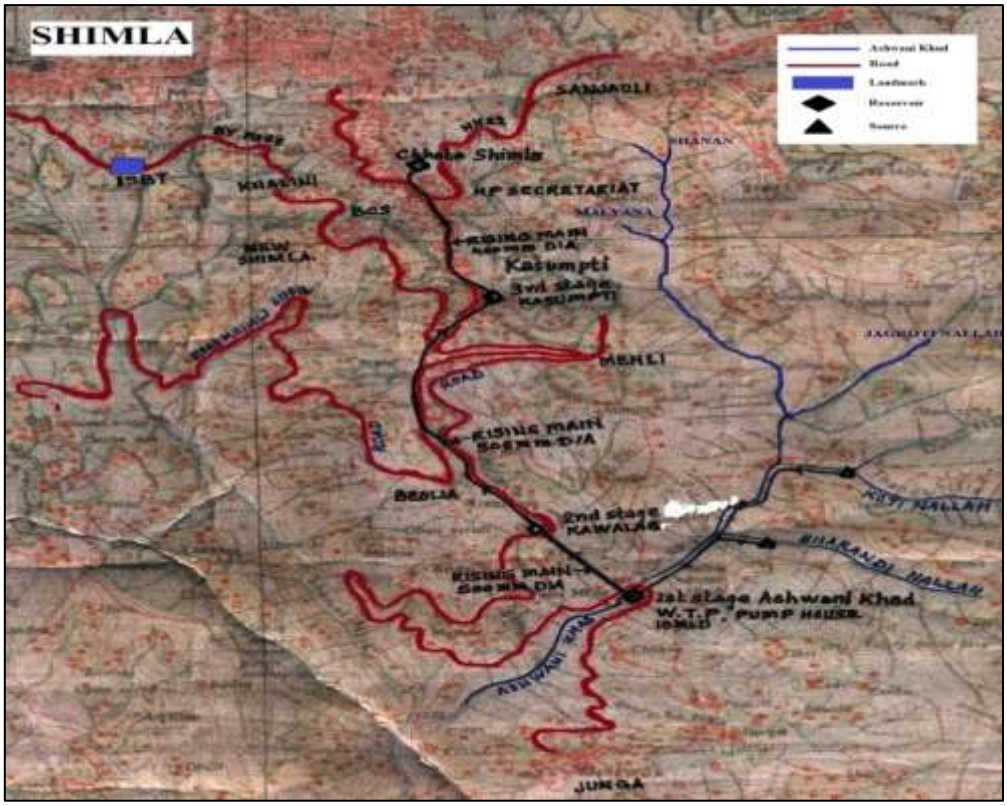


Fig. 4.10 Contributing tributaries of Ashwani Khud.

Out of these nallahas, the Malyana (STP) Nallah, Housing Board Nallah, and Sanan Nallah which originates from densely populated areas have some contamination. The water coming from these nallah has some chances of being contaminated due to outflow of numerous domestic septic tanks, solid waste, and STP treated water with Malayana (STP) nallah as major suspect having contamination.

In response to the jaundice outbreak due to sewage contaminated water, the source of Ashwani Khud was limited to Koti-Bharandi Nallah, the left hand side contributing tributaries of Ashwani Khud, decreasing the amount of water to be treated to 5.80 MLD with initial value at 10.06 MLD, about half of the treatment capacity of WTP.

Ashwani Khud WTP is designed to treat 10 MLD water. The water is treated through flocculator and sedimentation tank with the aid of alum and lime. Chemically treated water is filtered through rapid sand filter. Filtered water is blended with ground water and supplied to consumers after chlorination at WTP (clear water tank) and Kasumpati reservoir.

During design phase, chlorine dosing was planned at inlet which later on shifted to filter water tank. The process flow diagram is shown in the Figure 4.11.

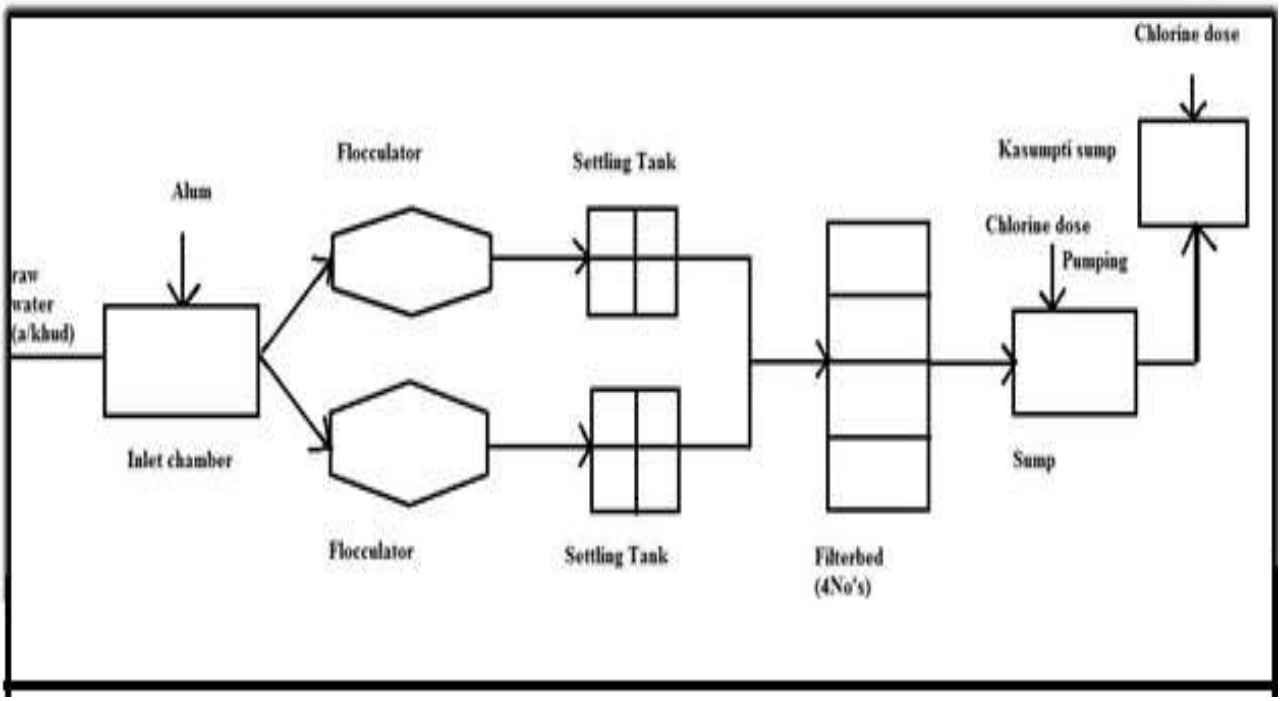


Fig. 4.11 Process flow diagram of Ashwani Khud WTP.

The Key Plan of Ashwani Khud WSS is shown in the Figure 4.12.

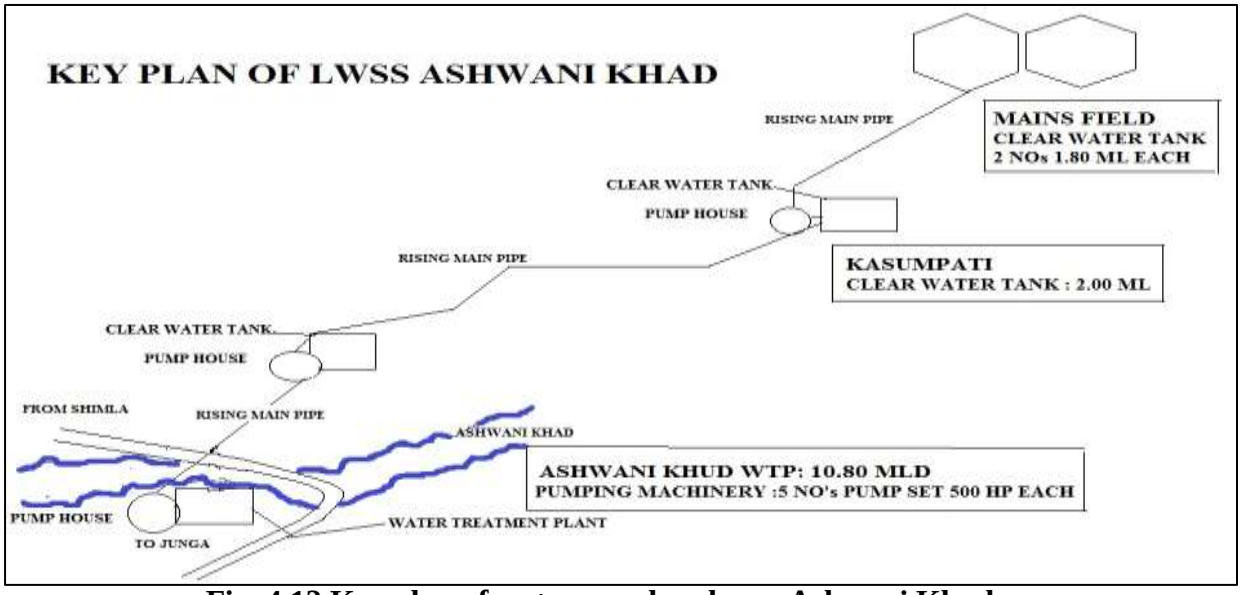


Fig. 4.12 Key plan of water supply scheme Ashwani Khud.

4.5 Physico-chemical characteristics

Physico-chemical charcteristics of various sampling points in the distribution line of Ashwani Khud (Koti-Bharandi) as a source was checked as explained in the section 3.3.

Results of different parameters tested are shown in the Table 4.9.

The values of pH and turbidity were found to be in permissible limit as per IS: 10500.2012. While the values of residual chlorine were found to be in permissible limit at the users end

i.e. tap water while the values at disinfection point and reservoir were much greater as it was measured just after the process of chlorination. But, the varying values of residual chlorine in tank 1 and tank 2, i.e. different tank outlets of the same clear water tank showed high chlorine demand of water. Thus, it was felt chlorination at further stages is needed to keep disinfection process in continuance to distribution processes to ensure pathogenic free water at the users end. At tap water the residual chlorine is found to be within permissible limit, thus fit for use.

Table 4.9 Physio-chemical characteristics of water samples.

Sample ID	Sample on 13-02-2017				Sample on 05-03-2017				Acceptable limit (IS:10500.2012)		
	T °C	pH	Turbidity	RC[a]	T °C	pH	Turbidity	RC[a]	pH	Turbidity	RC[a]
I	7	7.47	0.5	-	11	7.5	0.4	-	6.5-8.5	1-5	0.2-1
II	8	7.83	0.3	-	11	7.24	0.3	-	6.5-8.5	1-5	0.2-1
III	8	7.40	0.3	>4 0.5-1	10	6.97	0.3	2-3 0.5	6.5-8.5	1-5	0.2-1
IV	10	7.32	0.6	3-4	12	6.86	0.5	1	6.5-8.5	1-5	0.2-1
V	8	7.41	1.0	0.1	10	7.14	0.8	0.1	6.5-8.5	1-5	0.2-1

a= Residual chlorine

4.6 Microbiological characteristics

Microbiological parameters were studied and tested on the water samples taken from Ashwani Khud treatment and distribution system.

There were total ten numbers of samples, two from each sampling point, taken on February 13, 2017 and March 05, 2017 respectively. Result of each test is explained in the sections below.

4.6.1 Colony Forming units

The CFU or colony forming units were tested to check the availability of bacteria in the water samples procured from the various sampling points. The method and procedure for CFU determination is described in the section 3.5.1. Luria Agar is a media that encourages the growth of almost every type of bacteria. Thus, Luria Agar was selected as a growth medium for determining the concentration of bacteria present in water (if any).

In many cases where bacterial concentration is very high like one shown in figure 4.13, serial dilutions are made to simplify the counting process.

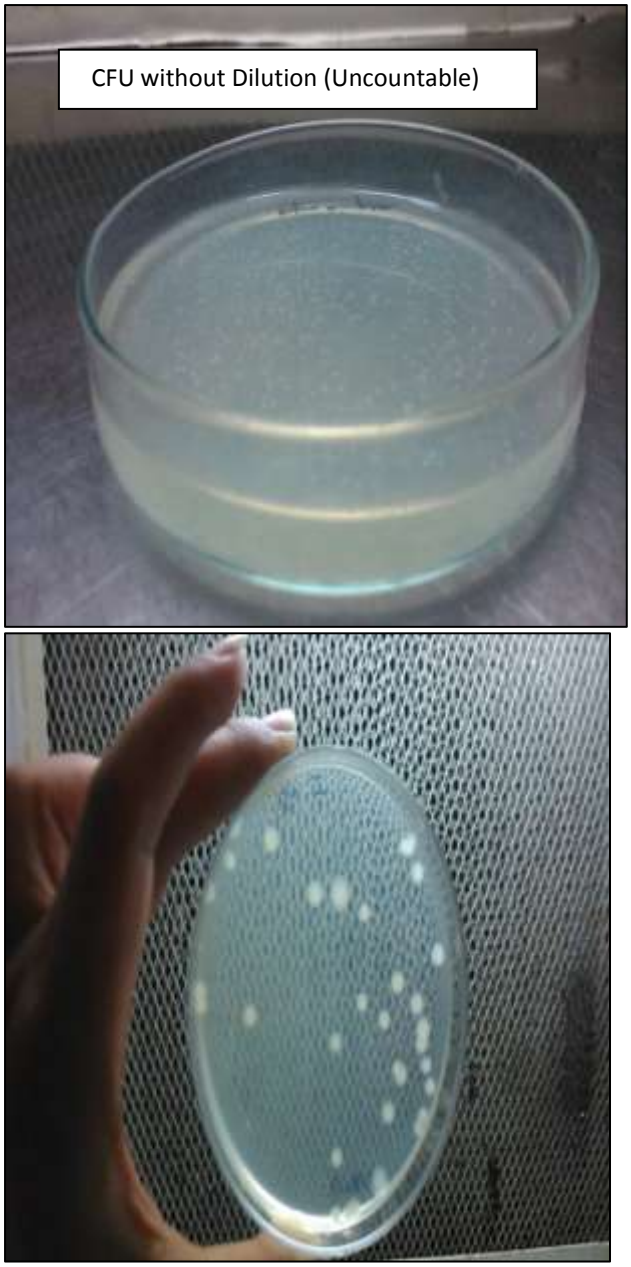


Figure 4.13 Variation of colony growth with dilution.

The CFU of various samples is shown in the Table 4.10, described in cells/ml.

Table 4.10 CFU of samples taken from listed sampling points.

Sample ID	CFU of sample taken on 13-02-2017 (cells/ml)	CFU of sample taken on 5-03-2017 (cells/ml)
I SOURCE	$5.0*10^2$	$1.36*10^3$
II BEFORE DISINFECTION	$2.5*10^2$	$5.92*10^3$
III AFTER DISINFECTION	$1.33*10^4$	$1.5*10^7$

IV RESERVOIR	$2.52*10^3$	$5.0*10^6$
V TAP WATER	$3.01*10^3$	$3.0*10^6$

Colonies grown on Luria agar after with/without dilution is shown in Figure 4.13. Few colonies were countable and if colonies formed were homogeneous and uniform, counted by making representative segments and then counted.

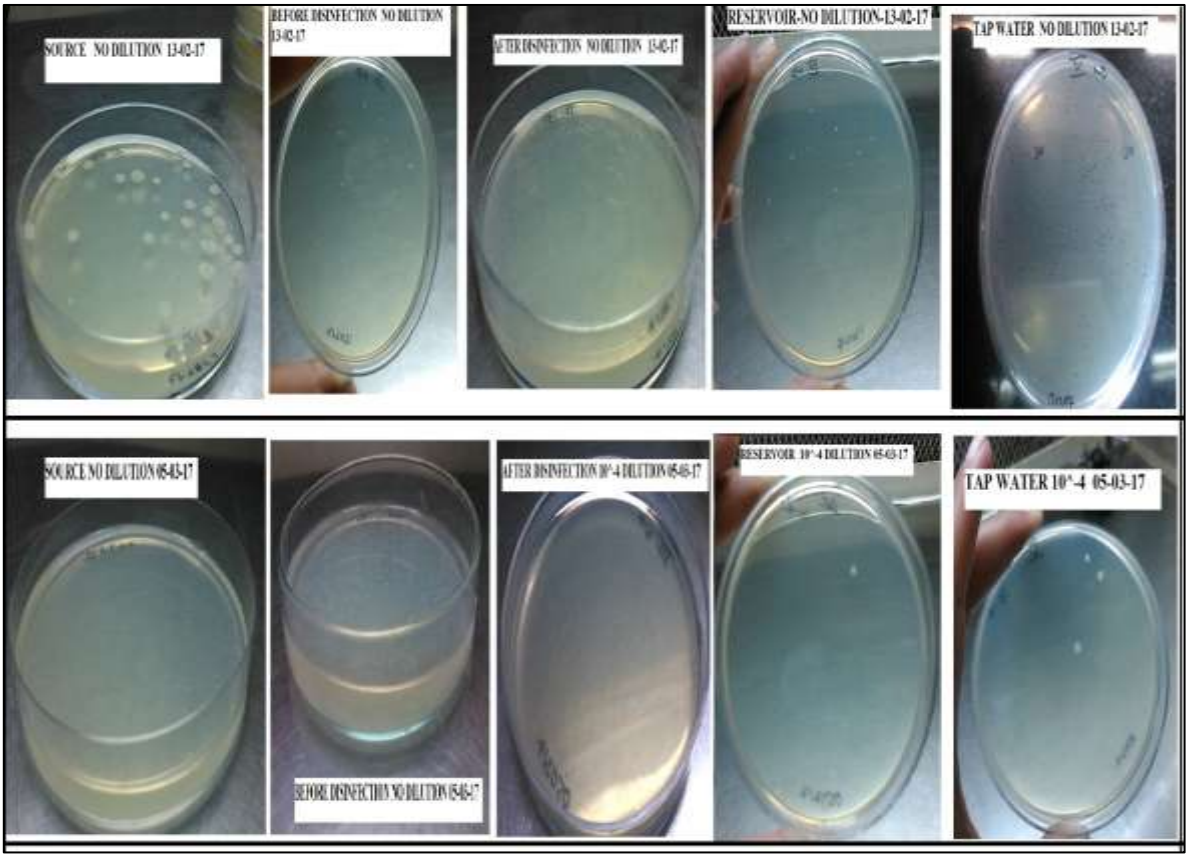


Fig 4.14 Colony formed on Luria agar by water samples.

4.6.2 Enrichment of water samples in media

Luria broth was used as enrichment media and water samples were inoculated in this media to check the growth and to further carry out the bacteria isolation processes.

The growth was measured in terms of turbidity attained by the media (broth) and amount of turbidity is denoted by (+) sign. Thus, values were allotted ranging from (+++) for highly turbid, (++) mild turbid and (+) little turbid media after incubation for the period of 24 hours at 37°C.

The results are shown in the Table 4.11 and Figure 4.15, respectively.

Table 4.11 Result of inoculation of the water samples in Luria Broth.

Sample ID	**Sample taken on 13-02-2017**	**Sample taken on 05-03-2017**

I Source	+++	+++
II Before Disinfection	-	+
III After Disinfection	++	++
IV Reservoir	++	++
V Tap Water	++	++

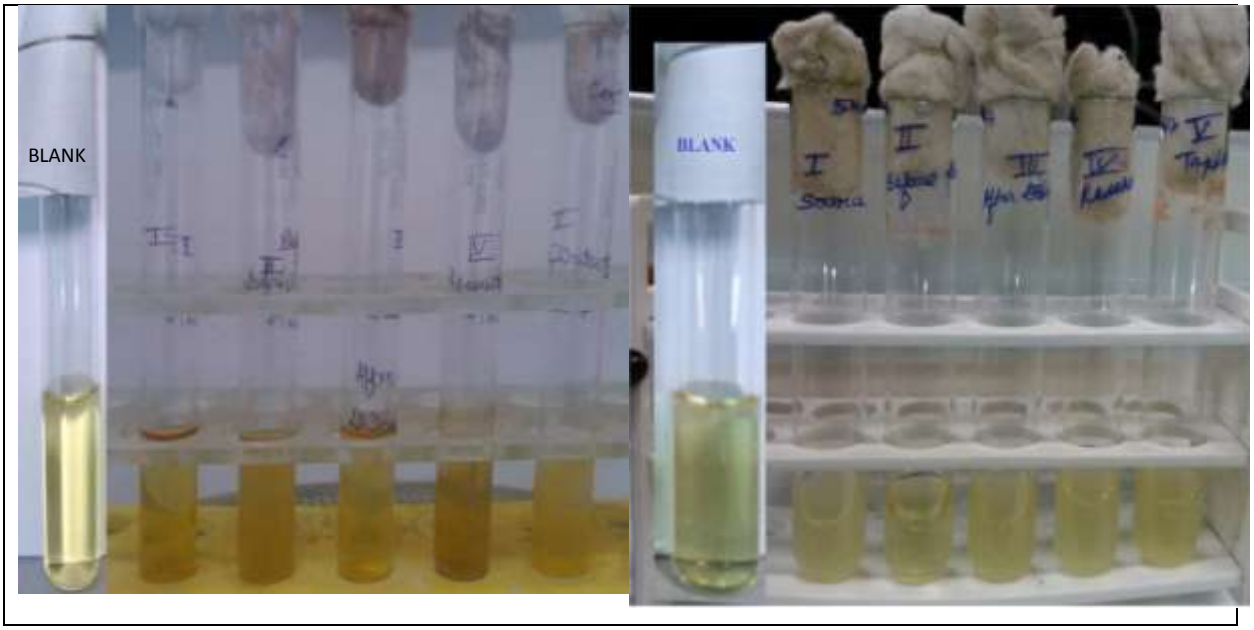


Fig. 4.15 Inoculation of water samples in Luria broth against a Blank.

4.6.3 Isolation of bacteria on selective media

After culture is grown in media, it is used for further isolation on selective media with the help of loop. The process is previously described in the section 3.5.3. The isolation results of cultures on Mac Conkey, XLD and TCBS are shown in the Figure 4.16 and Table 4.12

Table 4.12 Isolation of bacteria using method of streaking

Sample ID	Sample taken on 13-02-2017			Sample taken on 05-03-2017		
	MacConkey	XLD	TCBS	MacConkey	XLD	TCBS
I Source	LFC+++	WC+++ (1-1.5mm)	-	-	TSC (1-1.5mm)	-
II Before Disinfection	-	-	-	-		-
III After Disinfection	NLFC++	TSC(0.5mm)	-	NLFC++ (0.2mm)	TSC(0.5mm)	-
IV Reservoir	NLFC++	TSC(0.5mm)	-	NLFC++ (0.2mm)	TSC(0.5mm)	-

V Tap Water	NLFC++	-	-	NLFC++ (0.2mm)	-	-

Where:

LFC = Lactose fermenting colonies NLFC = Non-Lactose fermenting Colonies

TSC = Transparent small colonies WC = White Colonies

Growth on Mac Conkey: Lactose fermenting bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* produce acid, lowering the pH of the medium below 6.8, resulting in the appearance of pink colonies.

Non-Lactose fermenting bacteria such as *Salmonella*, *Proteus* species, *Yersinia*, *Pseudomonas aeruginosa* and *Shigella* cannot utilize lactose, but utilize peptone, further leading to formation of ammonia, which raises the pH. Thus, leads to the formation of white/colorless colonies on the agar.

Growth on XLD: *Pseudomonas aeruginosa*: formation of flat, rough colonies.

Growth on TCBS: is a type of selective agar that is used to isolate *Vibrio* spp. No growth means *Vibrio* spp are absent in the water samples.

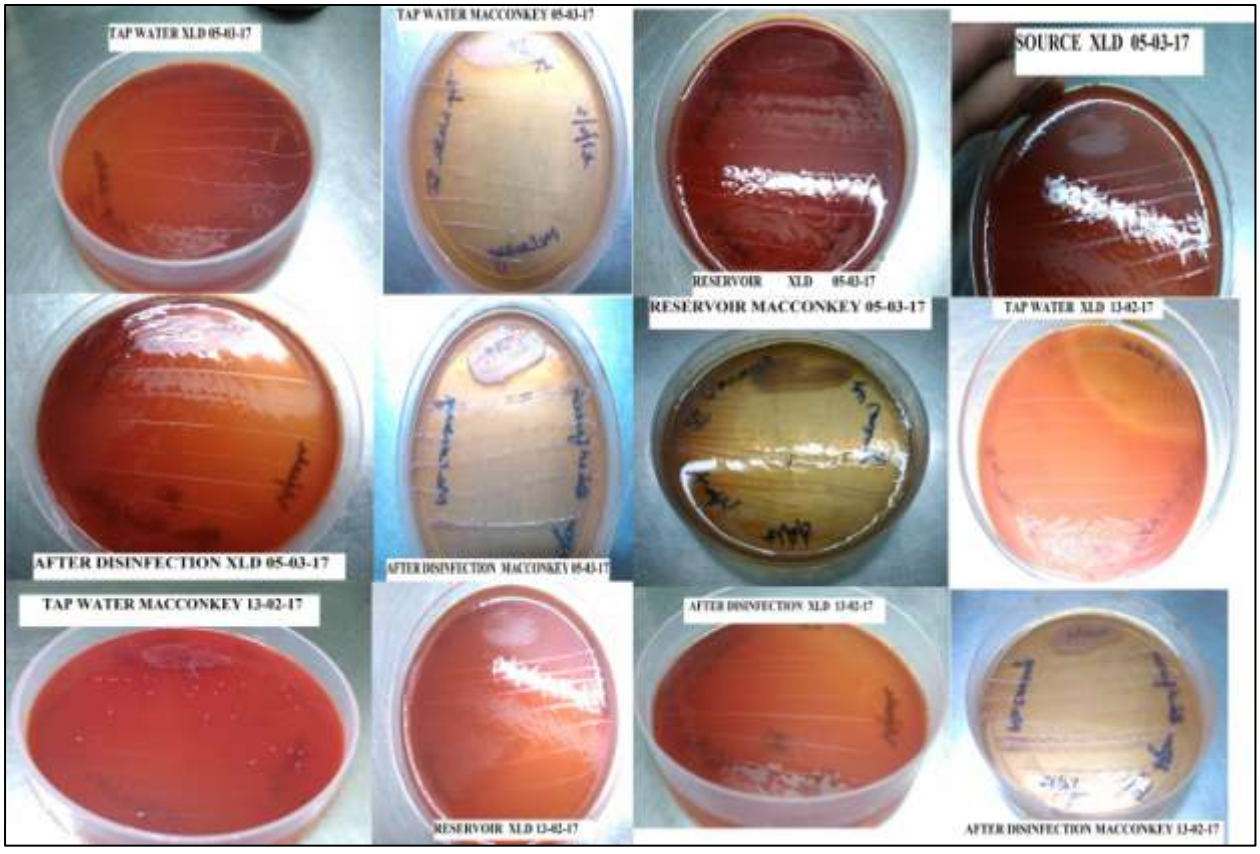


Fig.4.16 Isolation of bacteria on selective media.

4.6.4 MPN

Most probable number is very important factor in checking if there is recent fecal contamination in the water source. Fecal contamination causes the most of waterborne disease. Thus it is important to determine total coliform and indicator organisms of fecal contamination in water.

Most probable number was calculated using multiple tube fermentation technique as described in section 3.5.4.

The result is shown in the Table. 4.13.

Table 4.13 MPN/100 ml of water samples from various sampling points.

Sample 13-02-17	**No. of positive 10 ml tubes**	**No. of positive 1 ml tubes**	**No. of positive 0.01 ml tubes**	**MPN/100 ml**
I Source	0	0	0	0
II Before disinfection	0	0	0	0
III After disinfection	0	0	0	0
IV Reservoir	0	0	0	0
V Tap water	0	0	0	0
Sample 05-03-17	**No. of positive 10 ml tubes**	**No. of positive 1 ml tubes**	**No. of positive 0.01 ml tubes**	**MPN/100 ml**
I Source	0	0	0	0
II Before disinfection	0	0	0	0
III After disinfection	0	0	0	0
IV Reservoir	0	0	0	0
V Tap water	0	0	0	0

From the table above it is concluded that there was no fecal contamination in the water samples. Positive results in CFU and streaking prove that there are bacteria other than one from fecal contamination.

4.6.5 Biochemical tests

The biochemical tests were performed to identify the bacteria. IMViC tests were performed as explained in the section 3.5.5. The summary of all the biochemical tests performed is shown in the Table 4.19. The individual results of IMViC and TSI tests are discussed below:

4.6.5.1 Indole production test

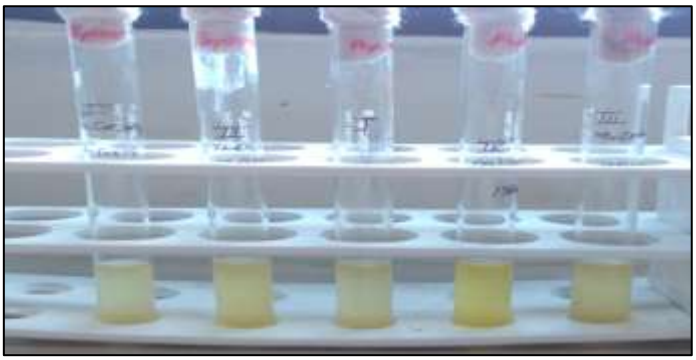


Fig. 4.17 End result of indole production test.

Figure 4.17 shows the indole production test result for the samples taken on 13 Feb 2017. Yellow ring formation is a negative while formation of red ring is a positive result. Table 4.14 shows indole production result of various samples.

Table 4.14 Indole production test results.

Sample ID	Sample taken on 13-02-17	Sample taken on 05-03-17
I XLD	-	-
I MAC CONKEY	-	N.A
III XLD	-	-
III MAC CONKEY	-	-
IV XLD	-	N.A
IV MAC CONKEY	-	-
V XLD	N.A	-
V MAC CONKEY	-	-

4.6.5.2 Methyl red test (MR)

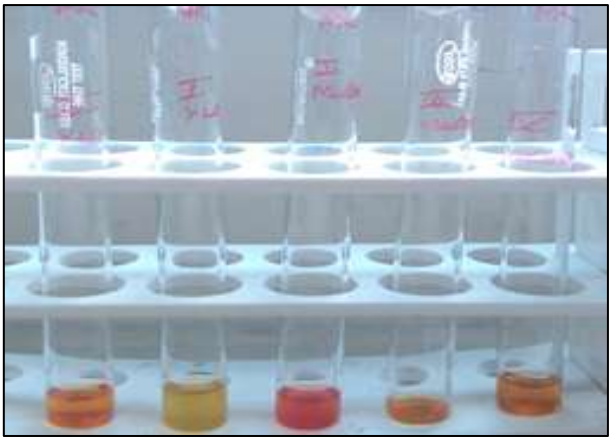


Fig. 4.18 Methyl red test on GPM cultured tubes.

Figure 4.18 shows result of the methyl red test on GPM cultured tubes for the samples taken on 13 Feb 2017.

Yellow ring formation is a negative while formation of red ring is a positive result. Table 4.15 shows Methyl red test result of various samples.

Table 4.15 Methyl red test result of various samples.

Sample ID	Sample taken on 13-02-17	Sample taken on 05-03-17
I XLD	-	+
I MAC CONKEY	+	N.A
III XLD	-	-
III MAC CONKEY	-	-
IV XLD	-	N.A
IV MAC CONKEY	-	-
V XLD	N.A	-
V MAC CONKEY	+	-

4.6.5.3 Voges-Proskauer test (VP)

Figure 4.19 shows result of Voges-Proskauer test on GPM cultured tubes for the samples taken on 13 Feb 2017. Black/grey ring formation is a negative while formation of red ring is a positive result. Table 4.16 shows Voges-Proskauer test result of various samples.

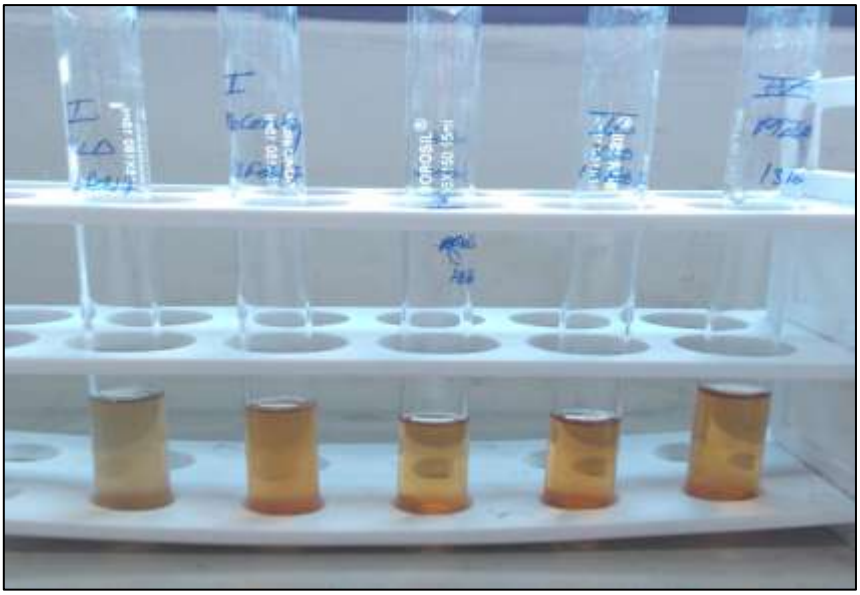


Fig. 4.19 Voges-proskaeur test on GPM cultured tubes.

Greyish-black ring formation is a negative test result.

Table 4.16 Voges-Proskauer test result of various samples.

Sample ID	Sample taken on 13-02-17	Sample taken on 05-03-17
I XLD	-	+
I MAC CONKEY	-	N.A
III XLD	-	-
III MAC CONKEY	-	-
IV XLD	-	N.A
IV MAC CONKEY	-	-
V XLD	N.A	-
V MAC CONKEY	+	-

4.6.5.4 Citrate utilization

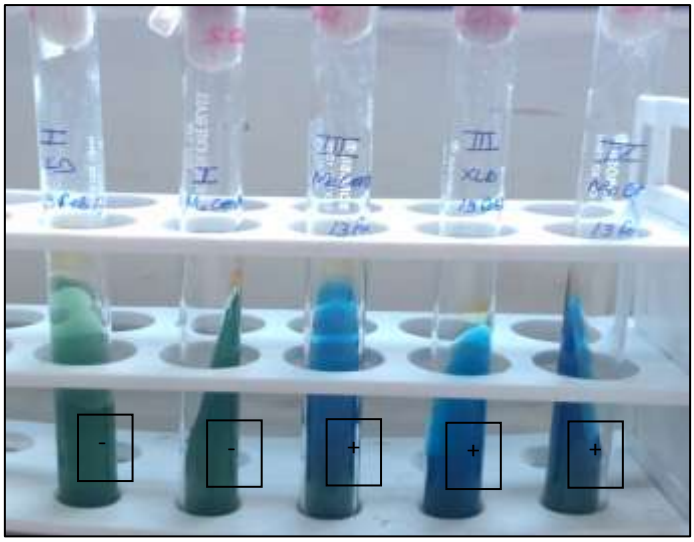


Fig. 4.20 Simmon Citrate cultured slant tubes result.

Figure 4.20 shows result of Simmon Citrate cultured slant tubes for the samples taken on 13 Feb 2017. Table 4.17 shows Simmon Citrate cultured slant tubes result of various samples.

Table 4.17 Simmon citrate cultured slant tubes result of various samples.

Sample ID	Sample taken on 13-02-17	Sample taken on 05-03-17
I XLD	-	+
I MAC CONKEY	-	N.A
III XLD	+	+
III MAC CONKEY	+	+
IV XLD	+	N.A
IV MAC CONKEY	+	+
V XLD	N.A	+
V MAC CONKEY	+	+

4.6.5.5 Triple sugar iron slant test

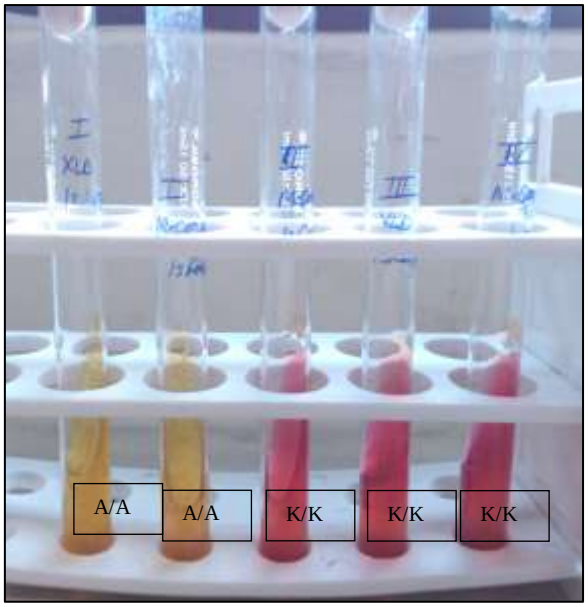


Fig. 4.21 TSI cultured slant tubes.

Figure 4.21 shows result of triple sugar iron agar cultured slant tubes for the samples taken on 13 Feb 2017. Table 4.18 shows triple sugar iron agar cultured slant tubes result of various samples.

Table 4.18 Triple sugar iron agar cultured slant tubes result of various samples.

Sample ID	Sample taken on 13-02-17	Sample taken on 05-03-17
I XLD	A/A	K/K
I MAC CONKEY	A/A	N.A
III XLD	K/K	A/A
III MAC CONKEY	K/K	A/A
IV XLD	K/K	N.A
IV MAC CONKEY	K/K	K/A
V XLD	N.A	A/A
V MAC CONKEY	A/A	A/A

4.7 Discussion

Variation of turbidity and residual chlorine with various water supply stages is shown in Figure 4.22 and Figure 4.23.

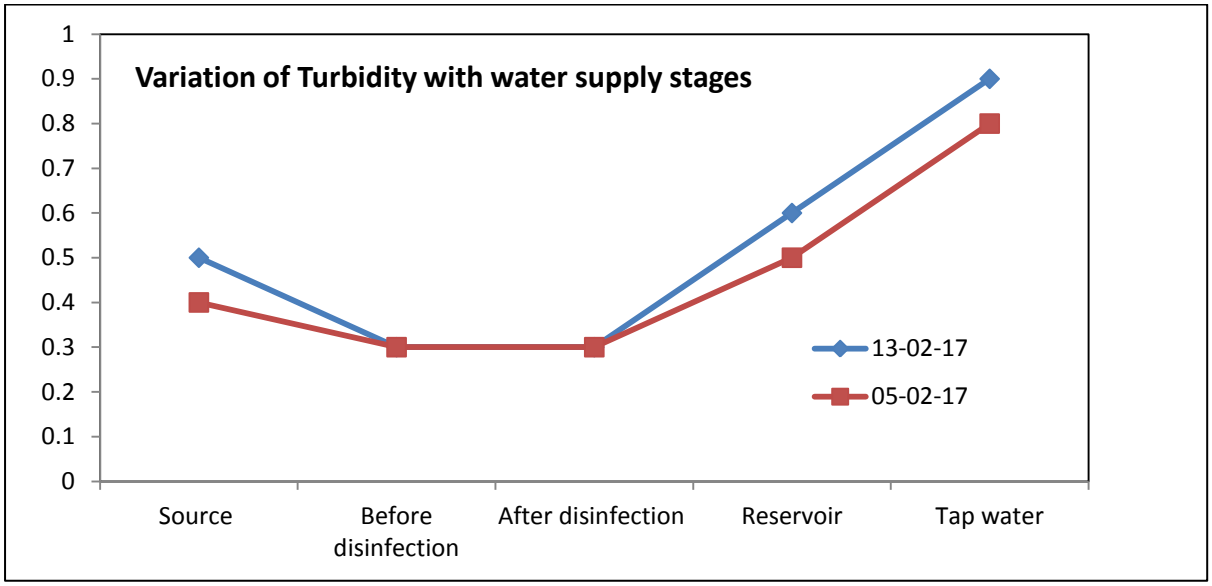


Fig. 4.22 Variation of Turbidity with water supply stages.

Figure 4.22 shows that filtration unit is working properly as ample amount of turbidity is getting reduced but after disinfection process, the turbidity is increasing exponentially till it reaches consumer at tap water. As we know, turbidity can reduce the effectively of disinfection and also might provide a surface for various microorganisms to grow (Garg. S.K.) thus, proper cleaning and maintenance of storage and distribution network should be done.

Residual chlorine, as shown in figure 4.23, the high variation of residual chlorine between tank 1 and tank 2, the different outlet of the same tank shows high chlorine demand of the water sample. This can be due to presence of high organics and high turbidity of the water. The value of turbidity is increasing in clear water tank, which also increases the chlorine demand of the water. Thus, chlorine demand is directly proportional to turbidity. Also, it is noted there is high turbidity in reservoir and tap water, so there is requirement of cleaning and maintenance of pipelines and storage tanks.

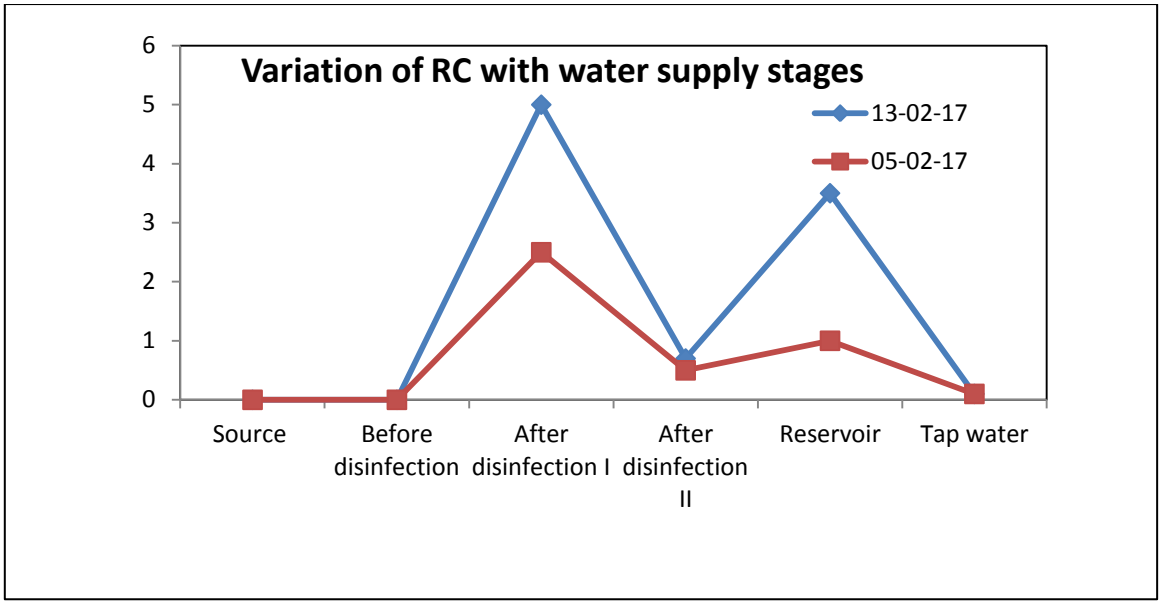


Fig. 4.23 Variation of Residual Chlorine with water supply stages.

CFU values of various sampling points is plotted and shown in Figure 4.24. CFU value showed that there is bacterial contamination in water samples. The value of CFU is increasing with source to clear water tank and decreasing after chlorination in the storage reservoir. This trend is also same in above two cases, i.e. the trend shown in figure 4.22 and 4.23 which also verifies the problem of contamination in clear water tank, reservoir and pipelines.

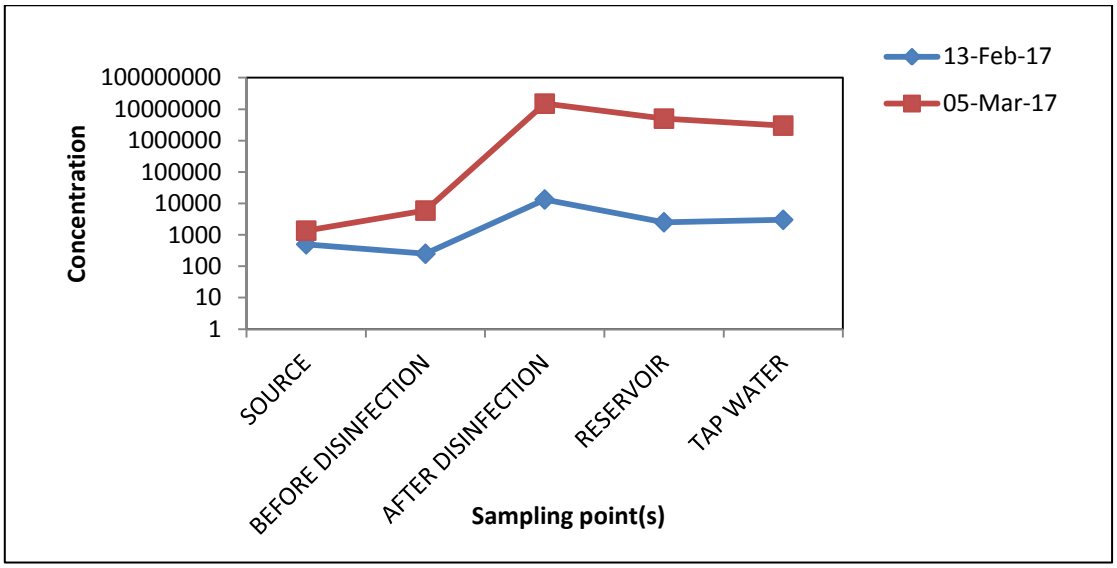


Fig.4.23 Variation of CFU during the different sampling periods.

As it can be seen in graph, the CFU value was greater in the month of March as compared to the month of February. This can be explained by the increase in temperature, thus providing favorable condition for microorganisms to grow. Also, the CFU growth flowed the same pattern, proving the study was reliable and there was some sort of contamination in water

samples just after disinfection was done till service reservoirs. We know there are many sources of bacteria be it soil, water or atmosphere (Ramesh,K.V).

The growth in enrichment media i.e. XLD and Mac Conkey, there were transparent and Non-lactose fermenting colonies respectively. No growth in TCBS proved there is no *Vibrio* species. The waterborne disease data also shows there is no Cholera outbreak confirming the reliability of test.

To check if there is fecal contamination in water, MPN test was performed, the result confirmed there is no fecal contamination. Thus, to identify the bacteria present in water sample, biochemical tests were performed.

The results obtained from IMViC tests are shown in figure 4.19. It shows that the bacteria in Clear water tank and reservoir during the sampling done on February 13 are same as they got similar pattern which resembles of Pseudomonas species. The bacteria in clear water tank and reservoir show similar pattern in IMViC tests during samples of March 05, which is of *Alcaligine faecalis.* It shows that there is some relation between clear water tank and reservoir, which makes the following two assumptions:

1. Contamination in clear water tank and its distribution in the water supply.

2. Contamination in both clear water tank and reservoir.

Chlorination is done in reservoir and clear water tank which shows the resistance of these species to chlorination. Thus, proper cleaning and maintenance of storage and distribution system should be done in order to remove the contamination/contaminants present in water supply system. Also, chlorination should be increase and other disinfectants or antibiotics should be tested to check their ability to reduce or terminate their growth.

Brief information of bacteria found in water samples

1. Streptococci are gram positive cocci arranged in chains or pairs, are part of the normal flora of human and animals. Some of them are human pathogens. The most important is *Streptococcus pyogenes* causing pyogenic infections, with characteristic tendency to spread. It is also responsible for acute rheumatic fever and glomerulonephritis, occurring sequel to

infection. Common diseases caused by them include upper respiratory disease infections, skin infections rheumatic fever, urinary tract infections (Paniker, C.K.J.,2005).

2. Alcaligenes faecalis refers to gram negative, short, non sporing bacilli,which are strict aerobes and don't ferment sugars. Alcaligenes faecalis is a saprophyte found in water and soil contaminated with decaying organic matter, also commensals in human and animal intestines. They are responsible for typhoid-like fever, urinary infections, gastroenteritis and suppuration in various parts of the body (Paniker, C.K.J.,2005).

3. Pseudomonas refers to large group of aerobic, non-sporing Gram negative bacilli. They are motile, ubiquitous, mostly saprophytic, being found in water, soil or other moist environments. A few of them are pathogenic to humans, causing human infection, typically opportunistic. It is one of the most troublesome agents causing nosocomial infections, with the most common is suppurative otitis, which is chronic though not disabling. In the hospital it may cause localized or generalized infections. Localized infections are common infections of wounds, eye infections and urinary infections. It is also one of the causes of infections in burns.

It has also been described as one of the agents responsible for infantile diarrhea and also been reported to cause a self-limited febrile illness (Shanghai fever) resembling typhoid fever in tropical areas. (Paniker, C.K.J.,2005).

Table 4.19 Summary of biochemical test result on different water samples.

Sample ID **13-02-2017**	**Indole**	**Glucose phosphate medium**		**Citrate**	**Triple sugar iron agar**	**Species**
		MR	**VP**			

I XLD	**-**	**-**	**-**	**-**	**A/A**	Not Identified
I MAC CONKEY	**-**	**+**	**-**	**-**	**A/A**	Not identified
III XLD	**-**	**-**	**-**	**+**	**K/K**	Pseudomonas spp
III MAC CONKEY	**-**	**-**	**-**	**+**	**K/K**	Pseudomonas spp
IV MAC CONKEY	**-**	**-**	**-**	**+**	**K/K**	Pseudomonas spp
IV XLD	**-**	**-**	**-**	**+**	**K/K**	Pseudomonas spp
V MAC CONKEY	**-**	**+**	**+**	**+**	**A/A**	Not identified
Sample ID **05-03-2017**	**Indole**	**Glucose phosphate medium**		**Citrate**	**Triple sugar iron agar**	**Species**
		MR	**VP**			
I XLD	**-**	**+**	**+**	**+**	**K/K**	Streptococcus spp
III XLD	**-**	**-**	**-**	**+**	**A/A**	Alcaligene faecalis
III MAC CONKEY	**-**	**-**	**-**	**+**	**A/A**	Alcaligene faecalis
IV MAC CONKEY	**-**	**-**	**-**	**+**	**K/A**	Not identified
V XLD	**-**	**-**	**-**	**+**	**A/A**	Alcaligene faecalis
V MAC CONKEY	**-**	**-**	**-**	**+**	**A/A**	Alcaligene faecalis

CHAPTER: 05

CONCLUSION

5.1 General

This chapter is dealing with the conclusion and salient findings of the research done. It thus, concludes the collected information, experiments performed and inference derived from them.

5.2 CONCLUSION

This research was based on analysis of water supply scheme of Shimla keeping in view waterborne disease outbreaks and the population affected by them. Studies concluded that prevalence of waterborne disease in Shimla was high and found to increase on yearly basis. Keeping the information collected on jaundice cases as a base, it was concluded that the zones served by Ashwani Khud water treatment plant, reported maximum jaundice cases. Also the weekly distribution of the jaundice showed it was common source problem. Thus, it was confirmed that the problem was at the Ashwani Khud water treatment and distribution system. Following jaundice outbreak and consumer complaints about sewage contaminated water at the household level from the zones suppled water by Ashwani Khud, the water suppy was suspended. Due to shortage of water and to meet high water demand the source of Ashwani Khud was restored with the supply being pumped from Koti- Bharandi. Thus, study was focused on Ashwani Khud as a source and its suitability as a permanent source of water to the major MC zones of Shimla. Water at the various stages of LWSS Ashwani Khud was analyzed for water quality analysis.

All the listed objectives as outlined in Chapter 1 have been achieved and important findings from the result are highlighted in this chapter.

Through the study of jaundice cases it was concluded that all the zones served by Ashwani Khud were at a high risk of infection due to contaminated water. So, need was felt to analyze its microbial water quality.

Physico-chemical water analysis concluded that parameters like pH, turbidity were within permissible limit (WHO, IS:10500) but low value of free residual chlorine was due to the presence of high organics in water source. Thus, proper cleaning and maintenance of storage and distribution system is recommended along with more chlorine dosage at the reservoirs to ensure pathogen free water.

Microbiological assessment of the raw water from Koti- Bharandi source concluded that the source water was free from any kind of fecal contamination thus proved fit as a source of

water supply. Fecal contamination was not found at any stage of water supply but other bacterial species such as Pseudomonas, Streptococcus and Alcaligene were detected at reservoirs and tap water respectively which showed their resistance to the disinfection processes.

By studying the treatment processes carried out at Ashwani Khud WTP, it was confirmed that the treatment processes were designed only for physical impurities but not for organics and contaminants (pathogens). Thus, it is recommended that the treatment processes must be upgraded in order to preserve water quality even cases when sewage is accidently mixed with drinking water source as happened in 2015.

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