Chapter Four
Microbiological Studies

Robert Koch for the first time used Environmental Microbiology to plan control of enteric microbial infections (Mitchell, 1992). Microbial monitoring is known to influence the water quality, in fact many epidemics take place due to slackness in monitoring microbial contamination. The basic purpose of microbial monitoring is to detect faecal pollution if any. Therefore microbial analysis is carried out to do real time monitoring of treatment and disinfection, as periodic examination, to uncover unsanitary conditions in the distribution system and to correct the causes of water borne disease outbreaks.

In developed countries access to safe drinking water is considered to be a fundamental human right. However, the definition of ‘safe’ is not much clear. It is generally assumed that adequately treated and delivered drinking water should not contain any pathogenic micro-organisms. As a practical objective, this is probably unattainable in most cases. In principle almost all enteric pathogens and opportunistic pathogens that are transmissible by the faecal-oral route can be transmitted through water.

This transmission particularly in the tropical developing country like India, due to the common open defecation practice in the rural areas as well as in bigger towns and cities, is very frequent. Thousands of individuals from poorer localities and children, even from economically not so poor families use open fields, roadsides for this purpose.

With increasing demands for water, as a result of population increase and changing life styles, the probability of contamination of surface and ground water by enteric bacteria is very common and is expected to increase even further. Water-borne outbreaks continue to occur as a routine matter in rural and urban conditions. A wide variety of enteric pathogens may occur in the drinking water supplies and wastewater in these areas.

Pathogenic bacteria that have been transmitted by water or wastewater include – *Salmonella, Shigella, Campylobacter, Enteropathogenic Escherichia coli (EPEC), Vibrio, Leptospira* etc.
Examination of routine bacteriological samples can not be regarded as a reliable measure of providing complete information concerning water quality. Bacteriological results must be considered in the light of information available about the sanitary conditions surrounding the sample source. Experience has revealed the significance of coliforms density as a criterion of the degree of pollution and thus of sanitary quality of the site. The importance of the test and the interpretation of the results have been well authenticated and used as a basis for standard of bacteriological quality of water supplies.

As discussed in the chapter one, contaminated water contains a variety of pathogens. Their number in water differs and their density usually much less than the 'indicator' selected. Thus making it very difficult to detect every pathogen in the water sample. Direct detection of pathogens is time consuming. In fact the tests involving indicators usually can be completed satisfactorily within reasonable time limits with greater reliability. Therefore it is more logical to select an indicator system and appropriate tests for the detection and enumeration of the indicator organisms rather than trying to detect specific pathogens.

An ideal indicator might be considered to possess the following characteristics

- It should be present in waste water and appear in polluted water whenever pathogens are present.
- It should not appear in unpolluted water.
- Indicator number should be greater than pathogen number.
- Indicator density should bear some relation to the degree or and extent of pollution.
- It should survive longer than the pathogens in water and have equivalent resistance to disinfectants.
- It should be applicable to all types of water for which microbiological criteria serve a purpose for public health.
- It should be detected by simple laboratory tests with reasonably high degree of accuracy in the shortest possible time.
- It should have constant characteristic.
Therefore it is clear that properties of indicator organisms should be such that their presence in water sample clearly indicates presence of other enteric pathogens.

These indicator organisms are constantly present in the contaminated waters. Their survival time is more than the survival time of other enteric pathogens, and they can be detected and confirmed within 48 hours. Therefore coliforms group of bacteria as herein defined, is the principle indicator of suitability of water for domestic, industrial and other uses. The cultural reactions and characteristics of this group of bacteria have been recorded elaborately (APHA, 1981).

It is customary to report results of coliforms test by multiple tube fermentation technique as Multiple Probable Number (MPN) index, which is also called as presumptive test for coliforms detection. When the indicator density is low (less than 1/100ml) the probability that a pathogen present in the sample is also very low. Samples giving these tests positive are further tested for confirmation of *Escherichia coli*, *(E.coli)* called as faecal pollution indicator.

*Escherichia coli*, a type of bacteria that grows in the colon of humans and other vertebrate animals and is typically found in water, is different from the sometime deadly strain that has been found in raw and undercooked meats. Health experts believe that waterborne *E. coli* is not a health hazard itself, but it indicates harmful faecal matter in the contaminated water.

Thus, a positive test for *E. coli* makes it more likely that the water is also tainted with harder-to-detect, other viruses like *hepatitis virus*, and enteric pathogens like *salmonella*, or the parasite like *gardiia* and *cryptosporidium*. Those parasites can cause diarrhoea or dehydration, and more serious illness in those with weakened immune systems. Even in the developed countries the problem of presence of *E. coli* is very severe as reported in the city water supply of Newyork (Newsday, 1995)

A large number of infectious agents, such as viral, bacterial, protozoan, helminthic and mycotic, in the faecal and urinary wastes of man and lower vertebrates are potentially waterborne. These can contaminate any raw source of drinking water or its distribution system very easily.
The problem of domestic waste is severe in and around Kolhapur city, and on many occasions people had horrifying experience of severe epidemic outbreaks of gastrointestinal diseases. The past data of water-borne diseases during the period of 10 years (1991-2000), was collected from the government hospital of Kolhapur (a much larger number of patients approaching private doctors was not available). This proved that the drinking water situation in the district was bad, and water might have contained almost all kinds of pathogens.

In Kolhapur city water purification and distribution systems are old, inadequate and outdated. A large population has to depend on other alternate sources of water, which may be equally poor in quality, for drinking and other domestic purposes.

The number of diarrhoea patients admitted in hospital showed an increase in the first eight years. Incidence of diarrhoea is always significantly more than dysentery and gastro-enteritis together. However except in the years 1992, 1993, and 1996 gastro-enteritis cases were more than dysentery. (Figure 20). Maximum number of cases 1246 were detected in 1998, followed by 1010 in 1997, and 991 in 1995 respectively. Similarly bacillary dysentery is also gastrointestinal disorder caused due to toxin production by some enteric and other pathogens present in food and water.

Diarrhoea is the main symptom of most of the infections by different pathogens listed in Table 1. It clearly indicates that many times these infections are caused by varied pathogens, which are found to be present in contaminated water. E. coli and some members of the paracolon group are inhabitants of the normal adult human intestinal flora, and are also important pathogens. Diarrhoea caused by toxin producing E. coli is the major cause of infant mortality in the developing world. (Stanier, 1989).

During present study continuous microbial monitoring was carried out for two year period from January 2000 to May 2002. For all types of drinking water samples, number of coliforms was estimated by multiple tube fermentation technique, standard plate count was taken and different pathogens detected. MPN index per 100 ml as
Year


Diarrhoea Dysentry Gastro

Figure-20 Number of cases of Diarrhoea, Dysentry and Gastroenteritis reported in Kolhapur in ten years (1991-2000)
well as SPC per ml was estimated at each site during the study which is given in Annex-1. Minimum, maximum and average values of MPN and SPC are expressed in Table 14. Correlation coefficient of these parameters is important and therefore these values are given in Table 10. After the isolation of bacteria on specific media cultural characters were studied. Biochemical characteristic study was carried out for the tests mentioned in the chapter two. Biochemical behaviour of the bacteria were studied in terms of enzymatic reactions and acid and gas production from different types sugars.

**Raw water samples (R1-R5)**

Raw water sources for Kolhapur city are Panchaganga river, Bhogawati river, and Kalamba tank. These sources revealed high values much above the prescribed limits of MPN and give values above 1800/100ml of water sample, (R1) with an average count 1139/100ml within 24 hours and 1234/100ml within 48 hours.

MPN values were found to be low in rainy season (55/100ml), and high (1800) in summer. Wide range of MPN index from 29 to 1800/100ml, was estimated during the study. Lowest index (29/100ml) in rainy season and highest (1600/100 ml) in summer was recorded. The average values were 692/100ml (24hrs), and 753/100ml (48hrs). Positive tubes of these presumptive tests were subjected further to detect faecal coliforms, that is *E. coli*, which was detected during entire course of study in these samples. Seasonal study showed that average values were high for both stations during summer season. Seasonal averages are given in Annex-2. Number of coliforms were low during rainy season, however, R1 had the count higher than R2 (Figure-21).

As discussed in the second chapter, Prayag being a confluence of two tributaries and a holy place, performance of rituals and religious activities is a routine practice at the banks of the river (Plate-I A). These samples showed higher MPN and SPC. Maximum and minimum SPC of R1 estimated was 5,78945 organisms/ml, and 458 organisms/ml respectively. Dilution of these microbial contaminants occur, therefore counts at R2 were comparatively lower. Maximum SPC 4,53,566 organisms/ml, minimum 231 organisms/ml, was estimated at R2.
Figure 20: Number of cases of Diarrhoea, Dysentry, and Gastroenteritis reported in Kolhapur in ten years (1991-2000)
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Figure-21 Seasonal variation in coliforms recorded at R1 and R2 during the study
Many times there is overflowing of Jayanti nala in the river during rainy season (Plate-I D), but the counts were lower because of the dilution due to floods. In summer season especially during electricity cut off when pumping of sewage was affected it entered in the stream and gave MPN values much above the limits.

Raw water is uplifted in respective filter houses, and subjected to treatment, before its distribution in the city. Inlet as well as outlet water samples collected on the same day were tested in the laboratory. MPN index estimated at R3 was in the range of 23 to 320/100ml, representing water quality of Bhogawati river, which was better than river Panchaganga. Number of coliforms were low in rainy season and high during summer. Inlet water samples of Balinga (R3) and Kasba Bawda water works (R5) showed higher counts in summer season, but Kalamba water works (R4) had higher MPN index during rainy season.

Among these three water works, Bawda filter house (R5) always had maximum number of coliforms during summer 802/100ml (Figure-22). R3 also showed higher count during summer (298/100ml). Maximum MPN index recorded at R4 during rainy season was (317/100ml).

Surface run off into the tank during rains is believed to increase coliforms, which is confirmed by the increase in the count during rainy season. The main problem of the city is Bawda water works, as its pumping station is 2 km downward of the discharge of Jayanti nalla. Its MPN varied from 5 to 1800/100ml, with maximum number most of the times in summer. Comparative analysis of MPN index of all raw water sources showed that Panchaganga has highest MPN (2400/100ml) at downstream (R5) where city domestic waste is discharged. Presently the water is used for irrigation purpose (Figure-23). R1 & R2 showed 1800/100ml as maximum MPN index. Lowest MPN index was estimated as 13/100ml (R4).

SPC was countable with maximum 541033/ml from Balinga filter house (R3). Average number of organisms were 99940 cell/ml. The lowest SPC estimated was 580/ml (R3). As per expectations bacterial count, which includes pathogens, and opportunistic pathogens, were high for R1, R2, R3 (Figure-24). When water sources for these three water works are compared then it is observed that R3 has maximum SPC (99,940 organisms/ml).
Figure-22 Seasonal Variation in coliforms during the three seasons at each filter house.
Figure 23: Minimum, maximum and average values of Coliforms from the raw water sources.
Figure-24 Maximum and average number of bacteria recorded in raw water samples R1-R5
Bacterial count was low for R5 giving average value 9781 organisms/ml even though high MPN index (729/100ml indicating contamination of sewage. Minimum SPC was estimated for R4, but *Flavobacterium remigenes* was detected in these samples, before as well as after treatment. Kalamba filter house is water treatment plant having Panchaganga as well as Kalamba tank as its raw water source. Large variation was observed in MPN index, which ranges from 13 to 540 coliforms/100ml. Many bacterial species were isolated on nutrient agar to study their cultural characters (Plate-VI A,B,C,D). However some bacterial species detected in each of the raw water sample during course of study are listed in the Table 11.

Table-11: Pathogens isolated from drinking water samples of Kolhapur

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia Coli</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Enterobacter aerogenes</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>4</td>
<td><em>Proteus mirabilis</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas fluorescence</em></td>
</tr>
<tr>
<td>7</td>
<td><em>Streptococcus fecalis</em></td>
</tr>
<tr>
<td>8</td>
<td><em>Staphylococcus auricus</em></td>
</tr>
<tr>
<td>9</td>
<td><em>Micrococcus roseus</em></td>
</tr>
<tr>
<td>10</td>
<td><em>Salmonella typhi</em></td>
</tr>
<tr>
<td>11</td>
<td><em>Flavobacterium rigense</em></td>
</tr>
<tr>
<td>12</td>
<td><em>Achromobacter fecalis</em></td>
</tr>
<tr>
<td>13</td>
<td><em>Citrobacter entermedis</em></td>
</tr>
<tr>
<td>14</td>
<td><em>Geotrichum candidum</em></td>
</tr>
</tbody>
</table>

Treated water and ESR sample (T1-T5)

A total of five samples were collected from filter houses and overhead tanks (ESR1 and ESR2) of the city. Destruction of the coliforms took place during chlorination process in the water treatment plants. Outlet water sample, collected
suddenly without prior intimation, showed high MPN index (200/100ml), this may be due to inefficient disinfection. It is very interesting to note that even after chlorination, outlet water sample of Bawda (T3) many times reported presence of coliforms, (2/100ml) with high counts of other organisms (45908/ml) including *citrobacter intermedius*, *flavobacterium rigense*.

The coliform bacteria were not detected in the outlet samples of Balinga water works (T1). Number of other bacteria present in the outlet samples are given in Table 14.

In overhead tanks (ESR), treated water remains for very short time period before distribution. The pilot study revealed that there was MPN count only from ESR of E ward, therefore water samples from only the two overhead tanks (T4 and T5) were analysed. ESR-1 (T4) did not have MPN, but house tap sample collected from a house located within 50 feet from this ESR, showed presence of faecal coliforms. There was also increased SPC. Water sample collected from ESR of Rajendranagar (T5) showed average MPN index 21/100ml. Pathogens, including *proteus mirabilis*, *Proteus vulgaris*, *E. coli*, *Enterobacter aerogenes*, were isolated from this sample.

**House tap water sample (HT1-HT10)**

House tap water is the end point of drinking water distribution network, which starts from water works. It was expected that no coliforms would be present in tap water samples. However, house tap of A ward (HT1), located in the bathroom of the house showed MPN index value of 4/100ml during summer season, with highest SPC 566230/ml. Fluctuations in the results of SPC were observed in house tap samples, the average being 98,997 organisms/ml. The species like *Achromobacter fecalis*, *Flavobacterium rigense*, *Citrobacter intermedius*, *E. coli*, were isolated from these samples.

In B ward a representative house tap in a bathroom having open drainage system was selected for study (HT2). This house was located in the campus of temple where devotees used this water, specially in the mornings (Plate ). For comparative study another tap located at a distance of 50 feet at the roadside was selected (HT3), and samples were collected on the same day. Water samples from HT2 showed MPN index of 2/100ml during summer, but HT3 did not have coliforms.
The pathogens *E. coli*, *Pseudomonas fluorescence*, *Proteus mirabilis*, *Streptococcus fecalis*, *Micrococcus roseus* were isolated from HT2. Coliforms were not detected from HT2. Microbial count of other bacteria was also low for HT3 (2364/ml) than 6665/ml at HT2. Pathogens including *E. coli* were found to be absent in HT3.

House tap located in bathroom of the house where all family members were using bathroom for most of the domestic activities (HT4) showed 54 coliforms/100ml during summer and average number of micro-organisms 6755 organisms/ml. Maximum number of micro-organisms were 23,750 cells/ml. While sometimes these organisms were found to be absent in the water samples.

From C ward (HT5) one house tap was selected which is located at the place where washing of cloths and large commercial utensils used for production of milk products, was carried out (Plate-V C ). The owner was not maintaining the cleanliness at this place, this was observed for three years during the period of study. HT5 showed MPN index above prescribed limit during summer (5/100ml) with 8 bacterial species out of total 14 species isolated during the course from 33 sites, were listed in Table 12.

Table 12: Pathogens isolated from C ward

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia Coli</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Enterobacter aerogenes</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Micrococcus roseus</em></td>
</tr>
<tr>
<td>6</td>
<td><em>Flavobacterium rigense</em></td>
</tr>
<tr>
<td>7</td>
<td><em>Achromobacter fecalis</em></td>
</tr>
<tr>
<td>8</td>
<td><em>Geotrichum candidum</em></td>
</tr>
</tbody>
</table>

From D ward only one tap was selected, located at roadside near drainage system (HT6). It did not had MPN index during two years of study. Many times it was observed that micro-organisms were absent in these samples. Other bacteria isolated were *Pseudomonas fluorescence*, *Micrococcus roseus*, and *Achromobacter fecalis*. 

65
From E ward 4 house taps were selected for comparative analysis. HT7 located in bathroom, HT8 located at clean place in an apartment, HT9 located 50 feet away from ESR-1 (T4) and HT10, 200 feet away from ESR-2 (T5). HT7 showed high MPN index 13/100ml than HT8 (2/100ml). HT9 showed presence of coliforms even though coliforms were totally absent in the water ESR-1(T4) sample. HT10 had MPN index value of 7/100ml. All pathogens listed in Table 11 were isolated from these house taps except *Salmonella typhi* which was not frequently present. Average count of bacteria was lowest in HT8 (52/ml) and highest (12542/ml) in HT9 sample.

**Public or common tap sample (CT-CT5)**

There are a large number of public taps of drinking water in Kolhapur city, (approximately 300). From the Kolhapur state days these public taps were maintained for steady and guaranteed drinking water supply for the needy masses. There has been significant increase in the number and distribution in these public taps in the last three decades. Presently these taps, located at different parts of the city, are managed by KMC. The number of these taps is more in B, A, and E wards, therefore samples of only these public taps from the wards were monitored during the study.

From ‘A’ ward a common tap (CT1) was selected as it was located near sanitary system. Coliforms detected (5/100ml) from the sample contained *E.coli, Pseudomonas fluorescense, Achromobacter fecalis, Micrococcus roseus, Flavobacterium nigense,* and *Citrobacter intermedius.*

Consistency was not found in the SPC results of these samples. The samples showed minimum SPC as compared to the house taps of other wards of the city.

SPC estimation showed extreme, as minimum value 0/ml and other extreme with maximum value of 23190/ml.

Public tap of B ward (CT2) was attached to large water tank located at the centre of the city. Water from the tank was used by roadside vendors for production of different food items, and also for drinking purpose by large resident population.

Water stored in this tank was distributed from Balinga filter house. There was complete absence of coliforms and count of other bacteria was also significantly low on many occasions, with an average number of (343/ml). *Micrococcus roseus,*
**Staphylococcus aureus**, *flavobacterium rigense*, *Achromobacter fecalis* were isolated from these samples.

From 'E' ward, three public taps were selected, (CT3) was located near public toilet, when monitored for microbial presence contrary to the expectations it was observed that even MPN index was low for these samples (2/100ml). Average SPC was 3,471 organisms /ml. Second sample (CT4) of public tap was collected from slum area (Plate-IVA) located at a distance of 50 feet from (CT3), showed highest MPN (1800/100ml) and 34701 organisms /ml as average SPC. It may be due to leakage in the pipeline system, as for CT4 it passed through the drainage system. Where as for CT3 it came from roadside to the tap, next to which public toilet is located (Plate-V A).

Public tap (CT5), located near drainage system was the third sampling point of this ward. MPN index was estimated only during winter season which was 2 and 5 per 100ml. SPC was 6114 organisms /ml of sample. All bacteria listed in Table 11 were present in these three samples.

**Groundwater sample (HG1-HG5)**

Water distribution is not uniform with respect to time and its amount supplied in five wards of the city. Therefore in many areas of the city ground water pumped with the help of hand pump is the main alternate source of drinking water.

Water contamination did not necessarily depended on depth of tube-well. Even deep tube-well had showed faecal pollution, which proved that sewage contamination in ground water had badly affected the city groundwater profile and posed a serious health hazard. Similar results were observed by some other workers during their study of groundwater. (Joshi, Verma 2000) During the course of study, eight water samples from hand pumps five (HG1-HG5) and bore taps three (TG1-TG3) were monitored.

E ward bore samples collected from municipal corporation hand pump (HG5) had MPN index value in the range of 5 to 1800/100ml, as lowest and highest MPN respectively. High count of micro-organisms ranged from 467 to 49875cells /ml, and contained some fungi (Plate-VI C) and all bacteria listed in the Table 11. From the
same area sample collected from a private bore tap (TG3) had a count 0 to 550/100ml, with lower SPC 350 organisms/ml, and average of 2375 organisms/ml.

If MPN index of water sample collected from hand pump and bore tap, were compared then it was observed that average value of MPN during summer was maximum for hand pump, and high MPN index was recorded during winter and rainy season for bore tap water sample. (Figure- 25).

In E, B, and D wards many times people used ground water for drinking purpose, therefore in these wards comparative study of hand pump water and motorised private bore taps was carried out. It was observed that bore taps showed lower MPN index than hand pumps in B, (Figure- 26 ) and E wards (Figure- 25 ), but in D ward controversial results were obtained (Figure- 27).

Bore tap water samples from D ward were contaminated, and showed lower MPN index value of 2/100ml and high MPN value of 230 /100ml. Minimum of 45/100ml and maximum 340 /100 ml coliforms were estimated from hand pump water samples. Maximum SPC was 45890 /ml for bore tap and 655432 /ml for hand pump. Ground water of C ward was highly contaminated and showed highest MPN index value of 1800/100ml. Estimated SPC value was 238700 /ml.

Ground water collected from hand pump of B ward had lowest MPN 5/100ml and highest 680/100ml, with highest 348900 SPC. Milk boys routinely used this water to wash their utensils in which they stored milk (Plate- V E). It was suspected that small part of the water might have been added in the milk to increase the quantity. Yeast Geotrichum candidum was isolated only from these samples. This organism is usually present in dairy waste. It appeared that the organism existed in the ground water as one major city dairy (Bharat Dairy) existed in the vicinity and used to discharge its wastes in the municipal drainage system. which proved that dairy waste was percolating under ground in this area.

Bore tap water sample of the same ward showed maximum MPN 170/100ml(Fig -26) and 654409 organisms/ml of sample. Ground water of A ward showed highest MPN index during summer seasons (450/100ml) with average of 80/100ml. Number of other bacteria were countable, values of which are given in Annex 1. Comparative study of five wards showed that ground water contamination
A. Enteric bacteria on Mac-conkey’s agar.

B. Pigmentation of bacteria.

C. Fungal growth on nutrient agar at room temperature.

D. Bacteria isolated from drinking water.

E. Reason of bacterial contamination.

F. Drinking water distribution valve.

PLATE-VI
Figure-25 Comparison of average Coliform values from handpump (HG5) and bore tap (TG3) of 'E' ward during three seasons.
Figure- 26 Comparison of average Coliform values from handpump (HG2) and bore tap (TG1) of 'B' ward during three seasons.
Figure-27 Comparison of average Coliform values from handpump (HG4) and bore tap (TG2) of 'D' ward during three seasons.
was more in C and E wards (Figure -28). Bacteria isolated from these five wards is listed in Table 13.

**Table 13: Pathogens isolated from ground water of the city**

<table>
<thead>
<tr>
<th>S No</th>
<th>Location of Sample</th>
<th>Type of ground water source</th>
<th>Bacteria isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A ward</td>
<td>Hand pump HG1</td>
<td><em>E. coli</em>, <em>Proteus vulgaris</em>, <em>E. coli</em>, <em>Proteus mirabilis</em></td>
</tr>
<tr>
<td>2</td>
<td>B ward</td>
<td>Hand pump HG2</td>
<td><em>Geotrichum candidum</em>, <em>E. coli</em>, <em>Pseudomonas fluorescens</em>, <em>Klebsiella pneumoniae</em>, <em>Enterobacter aerogenes</em>, <em>Achromobacter fecalis</em>, <em>Citrobacter intermedius</em>, <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>3</td>
<td>B ward</td>
<td>Bore tap TG1</td>
<td><em>E. coli</em>, <em>Achromobacter fecalis</em>, <em>Enterobacter aerogenes</em></td>
</tr>
<tr>
<td>4</td>
<td>C ward</td>
<td>Hand pump HG3</td>
<td><em>Proteus vulgaris</em>, <em>Proteus mirabilis</em>, <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>5</td>
<td>D ward</td>
<td>Hand pump HG4</td>
<td><em>Micrococcus roseus</em>, <em>E. coli</em>, <em>Pseudomonas fluorescens</em></td>
</tr>
<tr>
<td>6</td>
<td>D ward</td>
<td>Bore tap TG3</td>
<td><em>Proteus vulgaris</em>, <em>Proteus mirabilis</em>, <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>7</td>
<td>E ward</td>
<td>Hand pump HG5</td>
<td><em>Proteus vulgaris</em>, <em>Proteus mirabilis</em>, <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>8</td>
<td>E ward</td>
<td>Bore tap TG3</td>
<td><em>E. coli</em>, <em>Micrococcus roseus</em>, <em>Flavobacterium rigens</em>, <em>Citrobacter intermedius</em>.</td>
</tr>
</tbody>
</table>
Figure-28 Seasonal values of coliform in the groundwater of five wards of Kolhapur (HG1-HG5)
### Table-14 Maximum, minimum and average values of microbial parameters at each site.

<table>
<thead>
<tr>
<th>Location</th>
<th>MPN /100ml (48 hours)</th>
<th>SPC/ml (48 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>R1</td>
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</tr>
<tr>
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<tr>
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<tr>
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</table>
Out of the 33 samples 30 samples were contaminated with *E. coli*, 18 samples were contaminated with *micrococcus roseus, achromobacter fcalis, enterobacter aerogenes, pseudomonas fluroscence*. 17 samples were found to be contaminated with *proteus vulgaris, proteus mirabilis, flavobacterium rigense*. 16 samples showed presence of *citrobacter intermedias* and 15 samples showed *staphylococcus aureus, Klebsiella pneumonias* was isolated from 14 samples and salmonella typhi was isolated from 9 samples. Yeast *geotrichum candidum* was present in two samples (Figure -29).

As listed in Table 11 during the course of study 14 bacterial species were isolated and identified from 33 water samples. Pathogens like *shigella*, was detected only once is not included in the list. Some fungi and actinomycetes were also not identified at generic level only their presence was detected.
Figure-29 Distribution of 14 bacterial species in drinking water samples (n=33) in Kolhapur City.