CHAPTER II

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MATERIAL AND METHODS

2.1 Introduction

Ground water, a gift of nature is about 210 billion m^3 including recharge through infiltration, seepage and evaporation. Out of this nearly one-third is extracted for irrigation, industrial and domestic use while most of the water is regenerated into rivers. Central treatment of water and its distribution through pipelines has been major source of water in cities. Now a days ground water is a principal source of drinking water in rural areas and also for irrigation, but it has earlier been polluted or overused, making it non-potable or resulting in its depletion. Pesticides, fertilisers, excess salts, and agricultural runoffs and drainage water have poisoned most of the drinking water sources.

Water is mostly used for industrial, municipal and agricultural purposes. In order to ensure right quality and quantity of water use for these purposes it is extremely important to monitor water supply thoroughly taking various aspects into consideration. The control of water pollution necessarily requires qualitative measurement of water pollution as it contains number of impurities or pollutants. Hence different types of examinations of water such as physical, chemical and bacteriological are necessary before the water is used for any specific purpose.

The present study on ground water quality analysis was undertaken with the objectives of generating baseline data on the quality of ground water sources from rural environment and evaluating the impact of human activities on them.

2.2 Objectives

The present study was undertaken with specific objectives in mind. It was observed that there is no information on the ground water quality, particularly from the villages around the rapidly growing cities like Kolhapur. These villages over the years are in the process of loosing their character and being transformed into sub-urban centres without proper amenities or development plans. This adversely affects the quality of life

of people under the name of 'development' which in reality is unplanned 'growth'. Ground water being one of the vital natural resources has been studied in the present attempt with the following objectives:

- To evaluate the quality of ground water in four villages around Kolhapur city.
- To check physico-chemical parameters from selected locations.
- To study the microbial and heavy metal pollution.
- To study seasonal variations in ground water quality.
- To find out health hazards in the study villages, if any, due to microbial and chemical pollution of drinking water.
- To study people's reactions to drinking water conditions.
- To suggest mitigation measures, based on the study, for the problems related to ground water contamination.

2.3 Hypothesis

The study is based on certain hypothesis derived from literature survey, interaction with locals, media reports and discussion with research scholars and experts in the area. The hypothesis are as follows :

- The ground water as compared to surface water is safer for drinking purpose.
- Improper disposal of domestic sewage causes ground water pollution in villages
- Excessive use of fertilisers in agriculture fields causes ground water pollution.
- Improper disposal of industrial effluent contaminates ground water.
- Use of contaminated ground water at domestic level causes different health hazards, mainly due to the pathogens in water.
- Poor sanitation is the major cause of water born diseases in the villages.
- There is lack of awareness about water quality and its use in the local people

2.4 Study Area

The Kolhapur district is situated in the Krishna-Panchaganga river basins. The district lies between $15^0$43' and $17^0$17' North latitude and $73^0$40' and $74^0$42' east latitude. It has an area of 8254.7sq. km. It is surrounded by Sangli district to the north, Belgaum district of Karnataka State to the east and south and Ratnagiri and Sindhudurg districts to

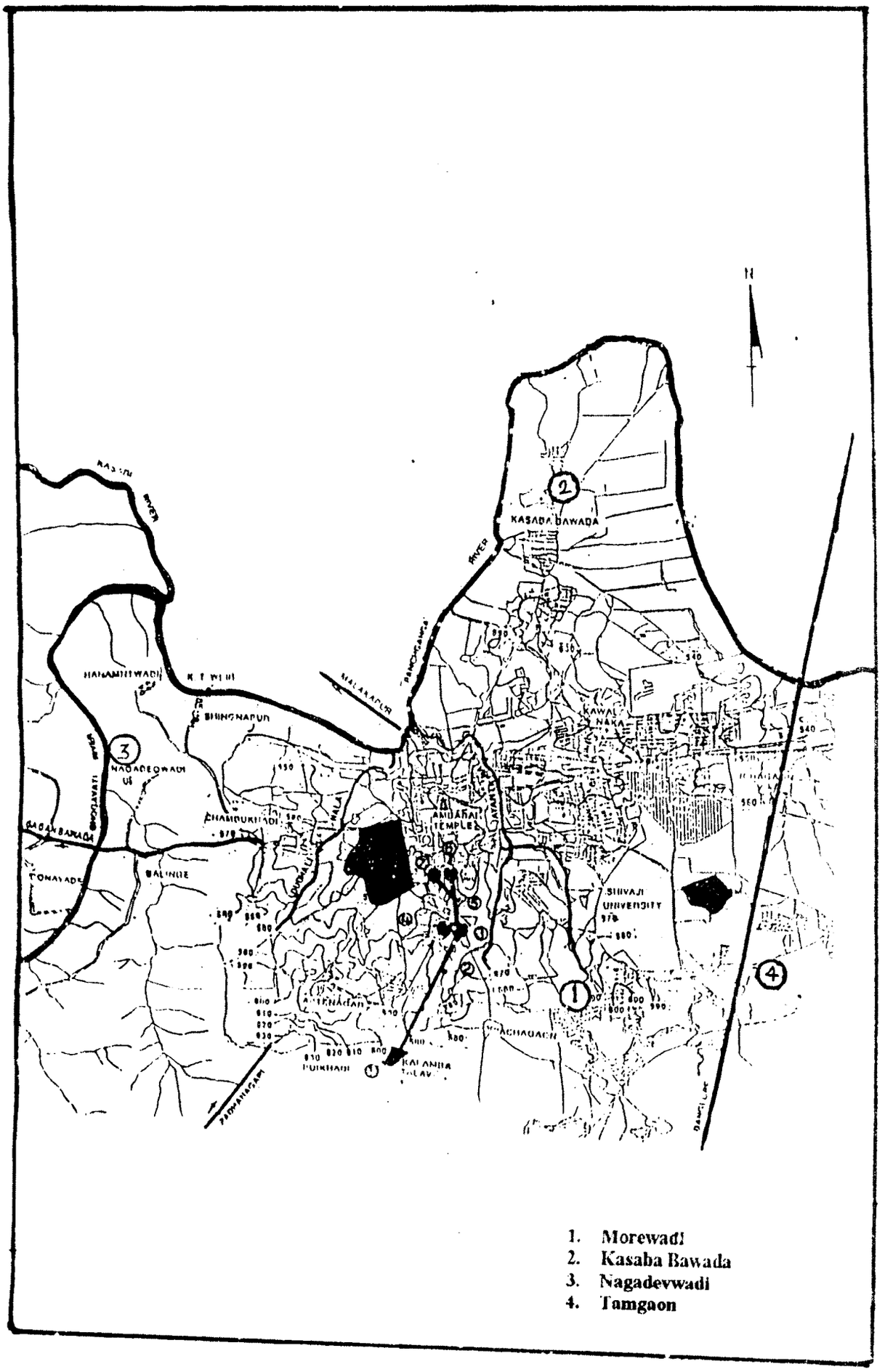


Figure 1: Location map of study sites in Kolhapur.

the west. The Sahyadri mountain ranges, with high rainfall, form the west and Warana river to the north form the natural boundaries. The district has an area of 7685 sq. km., which are about 2.50 % of the total area of the state.

Kolhapur, locally known as Karveer, is one of the oldest towns with a historical background of over 2000 years. The city now derives its importance from its past socio – cultural and political association and its present position as a great commercial, religious and educational centre. As religious centre, Kolhapur derives its appellation of Kashi of the south from the imposing ancient temple of Mahalaxmi. Educationally it has got importance, as it is a seat of learning with many educational institutions including Shivaji University. The city, basically residential, has two major industrial areas one at Shiroli and the other at Gokul Shirgaon. There are several sugar factories at some distance around Kolhapur City. Kolhapur is surrounded by agriculture areas dominated by sugarcane cultivation. The district has 12 towns and 1203 villages spread over 12 tahasils. Karveer has 128 villages situated in the tahasil

2.4.1 Profile of the study area

The area was initially surveyed for choosing the study villages, out of several villages around Kolhapur, after careful consideration given to various criteria of the study. The four villages finally selected, on the four sides of Kolhapur city, are namely Morewadi, Kasba Bawda, Nagdevwadi and Tamgaon belonging to Karveer tahsil. These villages were identified for the study on the basis of their representativeness in the region in addition to their specific locations and characteristics.

Table 3 Village profile of the four study villages (1991 census)

Name of village	Direction from Kolhapur	Total Area(ha)	Total population	Number of houses	Drinking water source
Morewadi	S	192.27	2525	510	Tap, well
Kasba Bawda	N	**	**		Tap , well
Nagdevwadi	W	246	2010	327	tap, river
Tamgoan	SE	804.27	4185	650	tap, bore well ,well

** the figures were not available as the village is partially merged in KMC limits

2.4.2 Climate

The climate of Kolhapur city is generally temperate. It is always cooler than the eastern part of the district which is liable to hot winds during April and May. The year may be divided into three major periods. Hot weather from March to May, rainy period from June to October and cold weather from November to February.

The district gets rain from the south-west as well as the north-east monsoons. The amount of rainfall received decreases rapidly from west to east. The south-west monsoon commences by about 1st week of June and lasts till about the end of September. The average annual rainfall within the district varies widely from about 600 mm (in Shirol tahasil) in the east to 6,000 mm (in Bavada tahasil) in the west. In Kolhapur city and around it is average 100 cm/yr

In winter, although day temperatures remain higher than the monsoon season. December and January are the coldest months of the year. There is rapid rise in temperature in March, reaching maximum in April and May. The pre-monsoon rainfall in this season accounts for about 10 % of the total annual rainfall.

2.5 Drinking water facilities in Kolhapur district

It is heartening to note that some drinking water facilities are existing in almost all villages and towns in Kolhapur district. However, It may also be noted that the issue of water availability has not been properly examined in depth and factors such as sufficiency or availability of water through out the year or otherwise, quality and portability of the available water itself has not probed into.

Traditionally the villages in the area depended for drinking water on surface water from rivers, tanks and open wells. Now in most of the villages open wells and hand pumps are the main sources of drinking water and in around 485 villages water is supplied by taps. Some villages still depend on river (n=476) and canal (n=5) for supply of water. It is found that around 813 (68.13%) out of 1,188 inhabited villages have more than one type of source of drinking water supply. Where as many as 82 villages in the

district reported single source of water supply viz. river, tank and spring or other than well, tap and hand pump.

The irrigated area in the district is distributed into 1,056 villages out of 1,188 inhabited villages accounting for 88.89%. In 26 villages irrigation is done by open well only. The area irrigated by river and well with electricity accounts for 70.78% and 21.15% respectively. The other sources used for irrigation are private canal (0.10%), open well (5.46%), tube well with electricity (0.03%), tank (0.04%) and others (2.44%). (District Census Handbook,1991). However, there has been significant increase in the supply of drinking water through taps in the recent years.

2.6 Methodology

The water samples were analysed for fourteen physico-chemical and three microbial parameters, at the nineteen identified sites from the four villages. The samples were collected during morning hours between 7.30 to 11.00 a.m., throughout the period of investigation. Heavy metals were studied ones during the study at all the sites. The sampling was done during the three major seasons for comparison purpose.

Selections of a sampling site, collection of sample, sampling frequency, preservation and analysis of water samples etc. was done by following standard procedures as prescribed by APHA and AWWA (1985) for drinking water monitoring.

2.6.1 Selection of sampling site

Selection of the sampling sites in the first place was decided on the basis of over all objective of the study.. The sites were carefully chosen after finalising the study villages. While selecting the sites in a village, the entire village was covered in the rapid pilot survey, interaction was made with the locals to identify the ground water issues and the number, location and type of the site was finalised.

Table 4. Water sampling sites in the five categories from the four villages

Sr. No.	Sampling site location	Code
	I) Hand Pump Category	
1.	Hand pump near a constructed gutter, R.K.Nagar, Morewadi .	HP 1
2.	Hand pump near house, open gutter, Kasaba Bawda .	HP 2
3.	Hand pump near road side, Kasaba Bawda .	HP 3
4.	Hand pump near garbage dumping, drainage, Kasaba Bawda .	HP 4
5.	Hand pump with proper hygienic conditions, Nagdevwadi.	HP 5
	II) Bore Well Category	
6.	Bore well near septic tank, R. K. Nagar, Morewadi	BR 1
7.	Bore well near toilet, drainage system, R K Nagar, Morewadi	BR 2
8.	Bore well water collected in tank(school)RK.Nagar, Morewadi	BR 3
9.	Bore well located near toilet, Nagdevwadi	BR 4
10.	Bore well near septic tank, Nagadevwadi	BR 5
11.	Bore well located near gutter , Tamgoan	BR 6
	III) Open Well Category (Plate III)	
12.	Open well in Krishividhyalaya, Kasba Bawda	W 1
13.	Open well at road side, in Tamgoan	W 2
14.	Open well in agricultural field, Tamgoan	W 3
	IV) Kolhapur KMC(KMC) Category	
15.	KMC water supply, R. K. Nagar, Morewadi	KMC tap1
16.	KMC water supply, Kasba Bawda	KMC tap2
17.	KMC water supply, Nagdevwadi	KMC tap3
	V) Grampanchayat Category (Plate III)	
18.	Grampanchayat water supply, Nagdevwadi	GP 1
19.	Grampanchayat water supply Tamgoan	GP 2

Plate - I

A) Hand pump located near a constructed gutter R.K. Nagar (HP1)

B) Hand pump located near house, open Gutter, Kasaba bawada (HP2)

C) Hand pump located near road, Kasba Bawada (HP3)

D) Hand pump located near garbage dump and stagnant waste water , Kasba bawada (HP4)

E) Hand pump with proper hygienic conditions , Nagadevwadi (HP5)

Plate - II

(A) Bore well located near septic tank. R.K.Nagar (BR 1)

(B) Bore well located near toilet & drainage system. R.K. Nagar (BR2)

(C)Bore well water collected in tank in school R.K. Nagar (BR3)

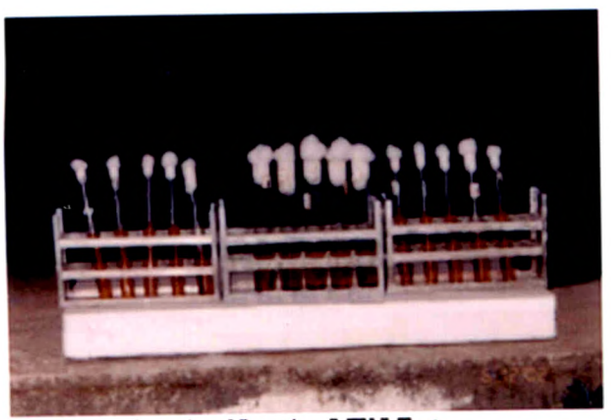
(D) Solid waste dumped in well in Krishi Vidyalaya, kasba bawda (W1)

(E)Well with depleted water level in summer, Tamgoan (W1)

(F) Lifted Well water used for domestic and agricultural purpose, Tamgoan (W2)

Plate - III

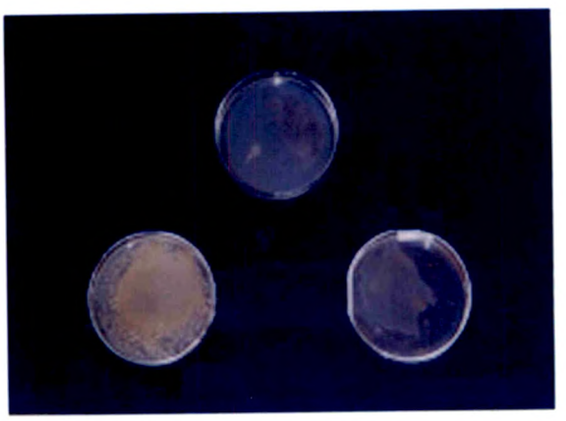
(A) Large queues for collection of drinking water at Grampanchayat in Tangoan .(GP 2)



(B) Photo showing Negative MPN Test

(C) Photo showing Positive MPN Test

(D) *Salmonella spps . isolated from drinking water*



(E) Standard plate count of bacteria

2.6.2 Frequency and number of samples

The frequencies and the number of water samples drawn depend upon the objectives, the source from where the samples were to be collected, time available for transport and analysis. The sampling frequency was determined considering the main three seasons and taking precautions to uniformly cover the entire study period.

2.6.3 Time interval between collection of water samples and analysis

The sampling programme was designed to provide the shortest possible interval between sample collection and analysis. Some of the parameters such as the determination of pH, temperature, dissolved oxygen, and other dissolved or evolving gaseous constituents are expected to be analysed on the spot as soon as the samples are collected. For rest of the chemical parameters, samples brought to the labcratory in well preserved condition, as early as possible, but in no case the time interval between the collection and beginning of the analysis, should exceed more than 72 hrs. The samples collected for bacteriological studies should be subjected to analysis within 24 hrs. During the present investigations this time limit was strictly adhered to.

2.6.4 Collection of samples

Samples were collected by using glass and polyethylene bottles. Samples for chemical and bacteriological analysis were collected separately. The minimum volume of sample required had been determined by the number of tests to be made and by the amount required for each test. Usually one litre of sample was sufficient. The glass containers for bacteriological samples were sterilised in an autoclave. All sample bottles were rinsed two or three times with the liquid to be collected before filling with final sample.

2.6.5 Sample preservation

If the samples can not be analysed within few hours of collection then some form of preservative treatment must be given to minimise chemical changes in the sample. Parameters such as temperature, pH, free carbon dioxide, alkalinity and dissolved oxygen were recorded at once. Dissolved oxygen was estimated by Winkler's iodometric

method; it was immediately fixed by adding Manganous Sulphate and alkaline Potassium Iodide solutions. The samples were brought to the laboratory as early as possible and kept in a freezer so as to check the biological activity and preserve them.

The water analysis was divided into three parts as- Physico-chemical parameters (n=14), 2) Heavy Metals(n=9) and 3) Bacteriological parameters (n=3). Total numbers of parameters studied were 26. The parameters studied in each category were as follows:

I) Physico-Chemical Parameters:

The fourteen physico chemical parameters studied from the potable water samples are 1) pH ,2) Temperature, 3) Electrical conductivity, 4) Turbidity, 5) Total Hardness in terms of $CaCO_3$, 6)Calcium(Ca^{++}),7)Magnesium(Mg^{++}),8) Free CO2, 9) Dissolved Oxygen (DO), 10) Total solids (TS), 11) Total dissolved solids (TDS), 12) Alkalinity, 13) Chlorides, and 14) Nitrates.

II) Heavy Metals:

The nine heavy metals from the drinking water samples studied during the study are 1) Copper (Cu), 2) Zinc (Zn), 3) Molybdenum (Mo), 4) Iron (Fe), 5) Cadmium (Cd), 6) Nickel (Ni), 7) Manganese (Mn), 8) Cobalt (Co), and 9) Lead (Pb).

III) Bacteriological Studies:

The bacteriological analysis of the drinking water samples was restricted to

1) Estimation of Most Probable Number (MPN) Test,
2) Standard Plate Count (SPC) technique, and
3) Isolation of *Salmonella* sp.

IV) Social Survey:

The social survey covered interviews of the locals from the four study villages namely Morewadi, Kasaba Bawda, Nagdevwadi, and Tamgoan by using interview schedule technique. A total of 57 individuals i.e. three schedules from each of the 19 sites were covered.

2.7 Description of Methods

Most of the parameters were analysed by using standard methods given by APHA AWWA and WPCF, (1985), for water analysis. The procedures adopted are briefly explained below.

I) Physicochemical Parameters:

1) pH:

pH is the measure of the intensity of acidity or alkalinity and measure the concentration of hydrogen ions present in water. It does not measure total acidity or alkalinity. There are many methods for determination of pH. During present investigation digital pH meter was used for measurement of pH.

1) Temperature (^{0}C) :

For determination of water temperature, water samples were collected in clean glass bottles. After collection of sample, a mercury bulb thermometer with 0.1 ^{0}C gradation was inserted in the sample and readings were recorded directly.

2) Electrical Conductivity (uMhos/cm):

The term conductivity denotes the response of a medium to the passage of current. Conductivity is a temperature dependent parameter, increases approximately 2 % for each degree-dissolved constituent and is therefore indicative of ionic strength.

Conductivity was measured in uMhos/cm using conductivity meter by using following formula.

Conductivity = observed conductance X cell constant X temperature factor at 25^{0}C

4) Turbidity (NTU):

The turbidity of sample is measured from the amount of the light scattered by the sample taking a reference with standard turbidity suspension, using Nephelometer. The value is measured in terms of Nephelomeric Turbidity Unit (NTU). Standard procedure given in APHA was calculated by using following formula.

Turbidity (NTU) = Nephelometer reading X 0.4 X dilution factor.

5) Total Hardness (mg/lit.):

Ethylenediamine tetraacetic acid (EDTA) titrimetric method was used for measurement of total hardness and the results are expressed in CaCO3 mg/lit.

$$\text{Total Hardness (mg/lit as } CaCO_3) = \frac{\text{ml of EDTA used x 1000}}{\text{ml of sample}}$$

6) Calcium (mg/lit):

Calcium was estimated by EDTA titration method using following formula.

$$\text{Calcium(Mg/lit)} = \frac{\text{ml of EDTA used x 400.8}}{\text{ml of sample}}$$

7) Magnesium (mg/lit):

Magnesium was estimated by EDTA titration method using following formula.

Mg (mg/lit) = [Total Hardness -Calcium Hardness] X 0.244.

8) Free CO_2 (mg/lit):

The amount of free CO_2 was estimated by titration method by using Phenolphthalein indicator.

$$\text{Free } CO_2 \text{ (mg/lit)} = \frac{\text{(ml of NaOH) x 1000 x 44}}{\text{ml of sample}}$$

9) Dissolved Oxygen (mg/lit):

There are many methods for estimation of DO. In the present study Wrinkler's iodometric method was used to estimate dissolved oxygen by using following formula.

$$\text{Dissolved oxygen (mg/lit)} = \frac{\text{(ml x N)of titrant x 8 x 1000}}{V_2\ (V_1\text{-}V/V_1)}$$

Where, V_1 = Volume of sample bottle after placing the stopper.

V_2 =Volume of the part of the contents titrated.

V =Volume of $MnSO_4$ and KI added.

10) Total Solids (TS) (mg/lit):

Total solids are determined as the residue left after evaporation of the sample and its subsequent drying in an oven at defined temperature and calculated by using following formula.

$$\text{Total Solids (mg/lit)} = \frac{(\text{Final wt.-Initial wt.}) \times 1000 \times 1000}{\text{ml of sample}}$$

11) Total Dissolved Solids (TDS) (mg/lit):

Total dissolved solids are determined as the residue left after evaporation of the filtered sample and calculated by using following formula.

$$\text{Total Dissolved Solids (mg/lit)} = \frac{(\text{Final wt.-Initial wt.}) \times 1000 \times 1000}{\text{ml of sample}}$$

12) Alkalinity (mg/lit):

The alkalinity was estimated by titration method by using methyl orange and phenolphthalein indicator.

$$\text{Phenolphthalein alkalinity as CaCO3 (mg/lit)} = \frac{(\text{A ml} \times \text{Normality}) \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}$$

$$\text{Total alkalinity as CaCO3 (mg/lit)} = \frac{(\text{B ml} \times \text{Normality}) \text{ of HCl} \times 1000 \times 50}{\text{ml of sample}}$$

13) Chlorides (mg/lit):

The chlorides were estimated by titration with silver nitrate solution and calculated by using following formula.

$$\text{Chlorides (mg/lit)} = \frac{(\text{ml} \times \text{N}) \text{ of AgNO3} \times 1000 \times 35.5}{\text{ml of sample}}$$

14) Nitrate (mg/lit):

There are many methods for estimation of nitrates. Phenol disulfonic acid (PDA) method was used during present study, by using UV visible spectrophotometer. Standard curve was plotted with different concentrations of nitrate to determine concentration nitrate in the sample.

II) Heavy Metal Analysis:

For heavy metal Analysis, initially acid digestion of water samples by using sulphuric acid and nitric acid was carried out, then metals were detected by Atomic Absorption Spectroscopy (AAS). The facility available in the Common Facility centre (CFC) of the university.

III) Bacteriological Analysis:

1) Most Probable Number (MPN) Test:

This is the presumptive test to estimate most probable number of coliforms. Coliforms constitutes all Gram negative, motile bacilli, facultatively aerobic in nature belonging to family *Enterobactiriceae* and able to ferment lactose at 37 ^{0}C ± 2 ^{0}C with production of gas. Multiple fermentation tube technique by using MacConkey's broth was used in Most Probable Number test.

To calculate MPN/100ml for various combinations of positive results when 5 tubes each of 10, 1 and 0.1 ml sample fractions are used.

a) MPN/100ml can be calculated by using Maccardys table.

b) In case of unlikely combinations following formula is used to calculate MPN /100ml.

$$\text{MPN/100ml} = \frac{\text{Number of positive tubes x 100}}{\text{Square root of total sample (ml) in negative tubes X total sample in test}}$$

2) Standard Plate Count (SPC):

It is a laboratory culture technique of determining the density of the viable aerobic and facultative anaerobic heterotrophic bacteria in water. The samples are inoculated on selected medium and incubated at 37^{0}C for 48 hrs.

Principle: Single microbial cell grows to form microbial colony. (Pelezar 1992)

For counting of most of the micro-organisms general purpose medium like nutrient agar can be used, on which micro-organisms are able to grow. This method also gives information about types of micro-organisms in addition to their number per ml of the analysed sample. Therefore this method was selected for the enumeration of micro-organisms. Number of micro-organisms per ml was determined by using following formula.

Number of micro-organisms/ml of water sample = No. of colonies x dilution factor x 10

3) Detection of *Salmonella* sp.:

For isolation of *Salmonella* four quadrant method was used. The media used for specific bacteria was specific. Bismuth sulphate agar, Wilson and Blair's medium and Brilliant green agar medium are routinely used for isolation of *Salmonella*. In the present study *Salmonella* was isolated by using Brilliant green agar medium.

The temperature used for incubation was 37^0C for 24 hours. Cultural study was carried out and the results were recorded in following manner. Colony Characters of an isolated colony on specific medium incubated at 37^0C for 24 hours were recorded as-size, shape, colour, margin, opacity, elevation, consistency, Gram nature and motility.

IV) Social Survey:

The perception of the locals about the drinking water problem in general and in their village in particular was to be understood. Attempt was made to study this aspect by conducting a social survey. For which standard stratified random sampling technique was used. The interview schedule was prepared by consulting the experts. Initially it was field tested in the pilot survey. Subsequently the schedule was improved as per the requirements and administered in the main study.
