CHAPTER-II
MATERIAL
&
METHODS
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Since growing up of the urban areas is very fast, are responsible for the deterioration of the natural resources, water and soil availability. Now, it is very difficult to get the clean, potable water and free environment in such areas, as well as the soil fertility faces tremendous threat. Because of increasing population and industrialization, the developments of the urban areas (urbanization/industrialization) is very essential but at the same time the quality of the water, soil and air (the environment) has also to be maintained for the survival of the life.

The present study was carried out from Waluj industrial area of Aurangabad District to evaluate the quality of the ground water. The aim of the study is to assess the impact of urbanization and industrialization and rapid growing developmental activities in the study area on the quality of ground water, soil, and to locate various sources and types of pollutants which are responsible for changes in ground water and soil quality. (Fig 19 to 28).

To assess the ground water and soil quality in Aurangabad region ten sampling stations (dug wells), which are scattered in the main areas of Waluj industrial area. The selected sites are of approximately 700 to 1000 m far from each other. The parameters used for the analysis of water and soil were located in industrial areas, creating certain interference in the surface water (soil) and ground water. In addition to water quality, certain heavy metals were also analyzed. Correlation on the quality of water in terms of physicochemical properties which is responsible for the water
Fig. 19: Groundwater pollution due to dumping solid waste in dug well. Waluj, Aurangabad.

Fig. 20: Groundwater pollution in the nearby dug well water and due to dumping solid waste. Waluj, Aurangabad.
Fig.21:- Ground water pollution in the nearby dug well water approximately 700 meters away. Waluj. Aurangabad.

Fig.22:- Groundwater pollution in the nearby dug well water & due to dumping solid waste. Waluj. Aurangabad.
Fig. 23: Disposal of industrial effluents in nearby stream its source from Waluj and Aurangabad.

Fig. 24: Disposal of industrial effluents in nearby field. Waluj. Aurangabad.
Fig. 25:- Disposal of industrial waste water in lake Waluj. Aurangabad.

Fig. 26:- Disposal of industrial waste water in natural lagoon Waluj. Aurangabad.
Fig. 27: Groundwater pollution in the nearby dug well water approximately 1 km away.

Fig. 28: Groundwater pollution in the nearby dug well water approximately 800 meters away.
pollution and since the health being a very important concept of community of the region, some indicator parameters such as MPN, E. coli were also determined to know the load of faecal matter in ground water.

Analysis of the samples was carried out seasonally throughout the years from summer 2006 to winter 2009. Each parameter was analysed seasonally. The data represented graphically and compared with the standards values laid down by WHO/ISI to determine the quality of the water for a particular sampling station and for a particular season.

In order to undertake accurate estimation of water quality, water and soil analysis were done for the parameters like temperature, colour, turbidity, electrical conductivity, total solids, pH, dissolved oxygen, Biological Oxygen Demand (BOD), Chemical Oxygen demand (COD), Total alkalinity, nitrates, phosphates, sulphates, chlorides, potassium, calcium, magnesium, total hardness, lead, copper, iron, zinc,..etc and E-coli and Most Probable Numbers (MPN). These analyses were carried out by referring the standard procedures according of APHA, AWWA, and WPCT, 1995, Trivedy, and Goel (1986) and NEERI. The results are expressed as ppm or milligram per liter.

In the study, the overall structure of the study area is taken into consideration while selecting the sampling cities.

The following steps were undertaken for the present study.
1. Selection of suitable ground water and soil sampling stations.
2. Collection of water, and soil samples,
   a) Laboratory analysis of collected samples by suitable (standard) method,
3. Evaluation of water and soil chemistry data obtained.
The following precautions were taken while collecting the water samples.

a) Plastic containers, resistant to the solution action of five liter capacity were used for the collection and storage of water samples.

b) Containers were thoroughly cleaned, acid washed and rinsed with distilled water before every collection.

c) For each station, a separate container was used.

d) The collected samples were labeled properly to indicate the location and date of sampling.

e) Water samples for dissolved oxygen were collected in BOD bottles (corning glass, 300 ml capacity while taking care) to prevent the formation of air bubbles, bottles were closed tightly under the surface of water.

f) The samples for chemical and bacteriological analysis were collected separately.

g) The measurement of temperature and pH were carried out on the field.

h) Analytical and other methods employed the type of investigation, the purpose of study, what data is needed and how it can be useful in a proposed study keeping all above pointes in view, the field and laboratory procedure were adopted to fulfill the objectives of study.

All the samples were protected from heat and direct sunlight during transportation until estimation.

Brief descriptions of the standard procedure are given bellow.
A) Physical Parameters:

Temperature:

Temperatures of the water changes with respect to depth, season and environment, also, many parameters like DO, BOD and COD are dependent upon temperature of the water sample. Temperature was measured in the field using a portable water quality analyzer and the results are expressed as °C.

The colour of water is found to be yellow or brown which occurs usually due to the presence of organic matter derived from soil, vegetation and its decay. It could also be due to metallurgical effluents. The colouring organic matter should, therefore, be removed from water and this can usually be achieved by the use of coagulants, in settlement tanks and passage through rapid sand filters.

Absence of turbidity in water sample is a prerequisite in the determination of colour, as it interferes in the measurements. Colour change is also caused by change in pH. So that results should be accompanied with the pH of the sample at which the colour has been determined.

The colour of water samples were quantified as absorbance at 367.5 nm using UV-visible spectrophotometer.

Conductivity:

Electrical conductance is an ability of solution water to conduct electric current in the presence of various ionic species. It is generally measured with the help of a conductivity meter, having a conductance cell containing two electrodes of platinum coated with platinum black of carbon. These electrodes are mounted rigidly and placed parallel at a fixed distance, conductance when measured between these electrodes having a surface area of 1 cm² and placed at
a distance of 1 cm is called electrical conductivity. It is the property of water samples, rather than that of the measuring system. The term specific conductance is also used in place of electrical conductivity, but it is an absolute term. The unit of conductivity is Siemens (S) Cm$^{-1}$, the older unit mhos cm$^{-1}$ is now rarely used. Conductivity of most waters is generally low and expressed in terms of us cm$^{-1}$. As ionization of solutes in water depends on temperature, conductivity results are reported at 25$^\circ$C.

**Total solids:**

Total solids were determined as the residue left after evaporation of the unfiltered water sample.

a) An evaporating dish made up of silica (100 ml capacity) was ignited at 550 ± 50$^\circ$C in a muffle furnace for about an hour. Then it was cooled in a desicator and weight was taken.

b) An aliquot of 100 ml unfiltered water sample was evaporated in the same evaporating dish on a hot plate maintaining temperature below 98$^\circ$C.

c) The residue was heated at 105$^\circ$C in oven for one hour and weighed after cooling the evaporating dish in a desicator.

Calculation

Total solid (mg/lit) = \((A-B) \times 1000 \times \frac{100}{V}\)

Where,

A-Final weight of the dish in g, B-initial weight of the dish in g,
C- Volume of the sample taken in ml.
B] Chemical Parameters:

pH:
The pH of sample was measured in the field using a portable water quality analyzer Kit (Century).

DISSOLVED OXYGEN (DO):

Dissolved oxygen in water was determined with the help of Winkler’s iodometric modified oxide method (APHA, 1995). In this method, addition of divalent manganese solution followed by strong alkali NaOH or KOH to water sample, rapidly oxidize manganese in the form of manganese hydroxide precipitate, giving an equivalent amount of dissolved oxygen present in water in the presence of iodide ions. On acidification, oxides of manganese revert to divalent state, with the liberation of iodine equivalent to original dissolved oxygen content in the sample. The iodine is then trapped with standard solution of sodium thiosulphate (0.025N). The sequence of reactions taking place is given below.

\[
\begin{align*}
\text{MnSO}_4 & \rightarrow \text{Mn}^{2+} \text{SO}_4^{2-} \\
\text{Alk. K} & \rightarrow \text{K}^+ + \text{I}^- \text{OH}^- \\
\text{Mn}^{2+} + 2\text{OH}^- & \rightarrow \text{Mn} (\text{OH})_2 \text{ white ppt. (O}_2 \text{ absent)} \\
\text{MnO}_2^+ + 2\text{OH}^- + \frac{1}{2} \text{O}_2 & \rightarrow \\
\text{MnO}_2 + \text{H}_2\text{O Brown ppt. (O}_2 \text{ absent)} \\
\text{MnO}_2^+ + 2\text{H}^+ + 2\text{l}^- & \rightarrow \text{l}_2 + 2\text{Mn}^{2+} + 2\text{H}_2\text{O} \\
\text{l}_2 + 2\text{Na}_2\text{S} & \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2 \text{NaL}.
\end{align*}
\]

An aliquot of 300 ml water sample was taken in BOD bottle to which 2 ml of manganese sulphate and 2ml alkaline iodide oxide solution was added, if brown precipitate is observed, dissolved oxygen is present, if white precipitate is formed, then dissolved oxygen is absent. The brown precipitate was dissolved by adding 2 ml
of concentrated $\text{H}_2\text{SO}_4$ and the same solution was titrated against 0.025 N $\text{Na}_2\text{S}_2\text{O}_3$ using starch as an indicator. At the end point initial dark blue colour turns to colourless.

The dissolved oxygen present in the water sample determined by using the following formula.

$$\text{DO (mg/l)} = \frac{\text{N of } \text{Na}_2\text{S}_2\text{O}_3 \times \text{xml of } \text{Na}_2\text{S}_2\text{O}_3 \times 8 \times 1000}{\text{V}_2 \times (\text{VI}-\text{V}) \times \text{VI}}$$

Where,

$\text{V}_1$ = volume of sample titrated. $\text{V}_2$ = total volume of sample,
$\text{V}$ = volume of reagent added.

**Biological Oxygen Demand (BOD):**

BOD is the amount of oxygen required by bacteria, while stabilizing decomposable organic matter under aerobic conditions. The decomposition of organic matter and metabolic activities of bacteria result in utilization of a part of the dissolved oxygen. This depletion of oxygen is considered as a measure of the amount of degradable organic matter in the sample under analysis.

Hammer (1977) gave details about the BOD test for polluted water and treated effluents. This test was performed for the samples from industrial effluents in the present area under study.

Water samples which were acidic or alkaline were neutralized to pH 7.0 with 1 N $\text{H}_2\text{SO}_4$/NaoH. Dilution water was prepared by aerating the distilled water till it became saturated with oxygen. Desired volume of distilled water was placed in a suitable bottle and one ml each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution and ferric chloride solution were added per liter of water. Several dilution of sample in the range of 0.1 to 1% was made to obtain required dilution.
Three bottles A, B, C each of 300 ml capacity, were chosen and filled with dilution water and stoppered without formation of any air bubble in them. Dissolve oxygen content of bottle ‘A’ was determined immediately. Bottle ‘B’ was used as a blank and in bottle ‘C’ 2ml effluent sample was added. These two bottles were incubated at 20°C for five days.

\[
\text{BOD (mg/l)} = \frac{(\text{initial DO} - \text{Final DO})}{\text{ml of water volume of BOD Bottles}}.
\]

**Chemical Oxygen Demand (COD):**

Trivedy and Goel (1986) suggested, chemical oxygen demand (COD) is the measure of oxygen consumed during oxidation of the oxidisable organic matter by a strong oxidizing agent potassium dichromate in the presence of sulphuric acid in determination of COD. The sample is refluxed with potassium dichromate solution and concentrated sulphuric acid in presence of mercuric sulphate to neutralize the effect of chlorides and silver sulphate (Catalyst). The excess of potassium dichromate is titrated against ferrous ammonium sulphate using ferroin as an indicator. The amount of potassium dichromate is proportional to the oxidisable organic matter present in the sample.

An aliquot of 20ml water sample was taken in 250ml of COD flask, 10ml of 0.25 N \(K_2Cr_2O_7\) solutions along with a pinch of silver sulphate and mercuric sulphate were added to it and aliquot of 30ml concentrated sulphuric acid was also added to the mixture. This solution was refluxed for 2 hours and then cooled to room temperature. The volume was made to 140 ml. from this 25 ml aliquot was used for titration, using 2.3 drops of ferroin as an indicator. The solution was titrated against 0.1 N ferrous ammonium sulphate until it
turned reddish brown from bluish green COD of the sample was estimated using the formula.

\[
\text{COD (mg/l)} = \frac{\text{ml of FAS} \times N \times 8 \times 1000}{\text{ml of sample titrated}}
\]

where, FAS is Ferrous Ammonium Sulphate. & N is the normality of FAS.

**Total Alkalinity:**

Larsen *et.al.*, (1955) and Thomas *et.al.*, (1960) have suggested different methods for the determination of alkalinity in natural waters in terms of equivalent of CaCO₃. Titrimetric method was therefore followed in the present work. Hydroxyl ions present in the sample as a result of dissociation or hydrolysis are determined by titration against hydrochloric acid using phenolphthalein indicator and hence, referred to as phenolphthalein alkalinity. The yellow colour changed to pink with methyl orange indication is called as methyl orange alkalinity. The total of both reading gives total alkalinity present in the water sample.

\[
\text{CaCO}_3 + 2\text{HCL} \rightarrow \text{CaCl}_2 + \text{H}^+ + \text{HCO}_3^-
\]

\[
\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{SO}_3
\]

\[
\text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2
\]

To 100 ml of water sample, two drops of phenolphthalein indicator were added; the solution remains colorless or turns pink depending upon the phenolphthalein alkalinity. If the solution turned pink after the addition of phenolphthalein, the mixture was titrated against 0.1 N HCl until end point when colour disappeared. This is termed as phenolphthalein alkalinity (PA).

After that, 2.5 drops of methyl arrange were added in the same mixture and titration was carried further until yellow colour changed...
to pink at the end point, which corresponds to total alkalinity (TA)
formula used for the calculations of total alkalinity is

\[
PA \text{ as } \text{CaCO}_3 \text{ (mg/I)} = \frac{A \times \text{Normality of } \text{HCL} \times 1000 \times 50}{\text{ml of sample}}
\]

\[
TA \text{ as } \text{CaCO}_3 \text{ (mg/I)} = \frac{B \times \text{Normality of } \text{HCL} \times 1000 \times 50}{\text{ml of sample}}
\]

Where,

\(A = \text{ml of HCl used with phenolphthalein.}\)
\(B = \text{ml of HCl used with phenolphthalein and methyl orange.}\)
\(PA = \text{phenolphthalein alkalinity.}\)
\(TA = \text{Total alkalinity.}\)

**Total Hardness:**

The hardness is normally expressed in terms of CaCO\(_3\). The EDTA method (Goetz, 1950, Goetz., Smith, R. C. 1959, Trivedy and Goel, 1986) was used to measure the concentration of calcium and magnesium ions in the water as a means of determining the total hardness of water. This method is based on the principle that Ethylene Diamine Tetra Acetic acid (EDTA) and its sodium salts form soluble complex when added in the solution of certain metal contains.

\[M^{2+} \text{EDTA} \rightarrow M-\text{EDTA complex}\]

A small amount of Erichome black T is added to an aqueous solution containing calcium and magnesium ions at the pH of 10. As a result, calcium and magnesium ion get complex and the solution become wine red. Since EDTA has strong affinity towards calcium and magnesium ions, on the addition of sufficient amount of reagent a complex of blue colour formed at the end of titration (Snoeyink *et. al.*, 1980). The chemical reaction which takes place in red and blue colour formation.
**Procedure:**

A 50ml aliquot of water sample was taken in a conical flask. To this, 1ml of buffer solution and 2.5 drops of Na2S solution were added, about 100-200 mg of Erichromate black-T indicator was added to the same, when the solution turned wine-red the mixture was titrated against standard EDTA solution. At the end point colour changes from wine-red to blue. The calcium and magnesium hardness was calculated using the following formula.

\[
\text{Total hardness (mg/l)} = \frac{\text{ml of EDTA used} \times 1000}{\text{ml of sample used}}
\]

**Total Dissolved Solids:**

Total dissolved solids were calculated by multiplying the observed electrical conductance of the sample measured by using digital conductivity meter (portable water quality analyzer), with Hem’s factor (Hem, 1970),

\[
\text{Total dissolved solids (mg/l)} = \text{E.C} \times 0.65
\]

**Nitrate:**

Nitrate ions react with brucine in strong sulphuric acid solution to form a yellow colour, which is measured spectrophotometrically.

Standard nitrate solutions in the range of 1 to 5 ml were placed in 50 ml beakers and diluted each to 5 ml. This included a beaker containing 5 ml distilled water, which served as the blank. One milliliter aliquot of brucine sulphanilic acid solution was added and mixed well, these solutions were transferred in a second series of beakers, already containing 10 ml sulphuric acid. Both solutions were mixed well and kept in dark for 40 min. After 10 minutes, 10 ml of distilled water was added to each beaker and allowed to cool in dark for 20-30 minutes absorbance of the standards were measured at 410
nm, after setting the blank at 100% transmittance. A calibration curve was prepared by plotting the absorbance against the concentration. The same procedure was followed for all water samples.

**Phosphate:**

There are several methods available for the determination of orthophosphate viz. Vandomolybdate method, molybdenum blue method and ascorbic acid method. Out of these, Vandomolybdate method was chosen in the present work for the estimation of phosphate in water samples. This method is considered to be slightly less sensitive than the molybdenum blue method but it has been particularly useful of phosphorus determination carried out by means of schoniger oxygen flask method. The phosphor vanado-molybdate complex formed between the phosphate, ammonium vanadate and ammonium molybdate is bright yellow in colour and its absorbance is measured at 465 nm.

A 10 ml aliquot (i.e. 4 mg phosphate) of this solution was placed in a 100ml graduate flask in the presence of 50 ml water and 10 ml of ammonium molybdate solution and diluted to mark. Like this, a series of standards were prepared from potassium dihydrogen phosphate standard solutions covering the range 0-20 mg phosphorus per 100 ml and containing the same concentration of acid, ammonium molybdate and ammonium vanadate as earlier absorbance of this solution was determined at 465 nm using 1 cm cell, against a blank prepared in the same manner. A calibration curve was constructed to find out the concentration of phosphate in water sample.
Sulphate:

The sulphate ion is one of the major anions occurring in the natural waters. Ingestion of water containing high concentration of sulphate has a laxative effect, which is enhanced when the sulphate is in combination with magnesium.

The sulphate was determined by the turbidimetric method. In this method, sulphate ions are precipitated in the form of barium sulphate by the addition of barium chloride in hydrochloride acid medium. The concentration of sulphate ion is determined from the scattering of light by barium sulphate and then by comparing it with standard curve using Mephlo – tubidimeter response in NTU

\[ \text{Ba}^{2+} + \text{SO}_4^{2-} \rightarrow \text{BaSO}_4 \]

In a series of volumetric flask 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml of standard sulphate solution was placed. A 10 ml aliquot of NaCl-HCl reagent and 20 ml glycerol-ethanol solution were added and made the volume to 100 ml. Each solution was taken out in a beaker and stirred on a magnetic stirrer. During stirring, 0.3 g of BaCl\(_2\) was added to each beaker and again stirred exactly for a minute. Turbidity was measured exactly after 4 minutes. The standard curve was prepared by plotting the response (NTU) against the concentration of sulphate ions in mg/lit.

Chloride:

Chloride concentration in the water determined by several methods viz argentometric or Mohr’s method, Mercuric method and potentiometric method. In present study, argentometric method was considered suitable for the determination of chloride ions. In neutral or alkaline solution, potassium chromate indicates the end point of titration of chloride. AgNO\(_3\), react with chloride ions to form very...
slightly soluble white precipitate of silver chlorite. After all the chloride is removed, the indicator changes its colour to reddish brown of silver chromate.

\[ 2\text{Ag}^+ + \text{CrO}_4^{2-} \rightarrow \text{Ag}_2\text{CrO}_4 \text{ (Reddish brown ppt)} \]

Water sample (30 ml) was taken in a conical flask and 2ml – \( \text{K}_2\text{CrO}_4 \) was added to it. The solution was titrated against 0.02 N \( \text{AgNO}_3 \). End point was taken when persistent red ring appeared, concentration of chloride (C1⁰) ions was determined using the following formula.

\[ \text{Cl (mg/I)} = \frac{N \times \text{ml of AgNO}_3 \times 35.5 \times 1000}{\text{ml of sample used}} \]

Where

N is the Normality of \( \text{AgNO}_3 \)

**Calcium:**

Volumetric determination of calcium was carried out by EDTA method. In this method, EDTA combines first with calcium and when pH is made sufficiently alkaline, magnesium is precipitated as hydroxide and the indicator muroxide combine colour turns to violet at PH 12 to 13. The concentration of calcium ions (Ca²⁺) is determined using the following formula.

\[ \text{Ca}^{2⁺} \text{ (mg/l)} = \frac{\text{ml of EDTA used} \times 400.8}{\text{ml of sample use}} \]

**Magnesium:**

The concentration of magnesium was estimated by subtraction the calcium content from the total hardness. Concentration of magnesium ions Mg²⁺ was calculated using following formula.

\[ \text{Mg}^{2⁺} \text{ (mg/l)} = \frac{(A-B \times 400.8)}{\text{ml of sample x 1.645}} \]

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Where,

\[ A = \text{ml of EDTA used in hardness determination} \]

and

\[ B = \text{ml of EDTA used in calcium determination}. \]

**Sodium and Potassium:**

Sodium and Potassium are present in a number of minerals. The increasing pollution of groundwater has resulted in a substantial increase in the sodium content of drinking water. The concentration of Na and K in waters varies from area to area. The contents of sodium and potassium in groundwater were estimated flame-photometrically, employing Elico Flame Photometer (Mode CL 22 D). The instrument was operated as per instruction of the manufacturer. The instrument was calibrated by using NaCl and KCl. The 10, 20, 50, 60, and 100 ppm standard solution were prepared for sodium and potassium.

**C] Heavy Metals:**

For analysis of heavy metals in water, samples were collected from the study area in three different seasons. Water, samples were collected in plastic containers, which were thoroughly cleaned with nitric acid and rinsed several time with distilled water. Analysis was carried out to determine the concentration of various metals like zinc, cadmium, copper, lead, flouride and iron by using atomic absorption spectrophotometer (AAS) (Alan Walsh, 1950’s). As it is the most versatile instrumental technique for the quantitative determination of trace metal in liquids. (Willard, Merrytt, Dean and Sattle, 1986). This method provides a fetal metal content of the sample and is independent of the molecular form of the metal in the liquid.
Versatility of AAS can be realized from the fact that 70 elements, including most of the common rare earth metals, have been determined by it in concentration that range from trace to macro quantities, in the presence of other elements.

Absorption of energy by ground state forms the principle of AAS, when a solution containing metal species is introduced into the flame. Some of the metal atoms may be raised to an energy level sufficiently high to emit its characteristics radiation, while other metal atoms remain in the non-emitting ground state. These ground state atoms of a particular element are receptive of light radiation of their own specific resonance wavelength (the same wavelength as they would emit if excites). Thus, when the light of this wavelength is allowed to pass through a flame having atoms of the metallic species, part of that light will be absorbed and the absorption will be proportional to the density of the atoms in the flame. Thus, in AAS, one determines the amount of light observed. Once this value of absorption is known, concentration of metallic, element can be determined.

The collected water sample acidified with concentrated HCl. Addition of acid not only destroys organic matter, but also brings all metallic compounds in suspension into solution. Aqueous samples were filtered and concentrated 5 times as per the procedure outlined by Handa (1986). Analyses of heavy metals such as Cu, Pb, Fe, Zn, etc were carried out in the present work.

For the elemental analysis, each sample has to be aspirated in the flame, having a fuel mixture of acetylene and air. The radiation from the hollow cathode lamp passes through this flame. The metallic
compounds are decomposed in the flame and the metal ion is reduced to the elemental state, forming a cloud of atoms. These atoms absorb a fraction of the radiation in the flame.

The decrease in radiant energy increase with the concentration of sought element in the sample, according to Beer’s law. The resultant radiation energy (light beam) passes through a slit to monochromater to eliminate extraneous light resulting from the flame and finally to detector and recorder.

To avoid errors, a blank solution (triple glass distilled water) is run before and after the aspiration of each sample into the flame. A separate lamp is used for the determination of each element.

D) Biological Parameters:-
Most Probable Number (MPN):

Multiple tube method-water receiving human and animal waste forms the primary source of water borne diseases, particularly in urban areas. Direct testing procedure, capable of detecting, qualifying the full spectrum of pathogen and identifying their sources of contamination are time consuming of their variable origin occurrence and survival rate. Hence, an indicator system, M P N, has been widely recognized the world over as the best method for evaluating the microbiological quality of water.

Coliform, especially fecal coliform test is considered as the most reliable test for detecting the contamination of fecal matter in the water. The coliform group comprises of “all aerobic and facultative anaerobic Gram negative non sporulating, rod shaped bacteria which ferment lactose to generate gas within 48 hours at 35°C”. Coliforms include most common intestinal bacteria, E.Coli less common pathogens, enterobacter, aerogenes. other common
pathogens include in this class are salmonella, shigella, streptococci, aeromonas etc.

Coliform density can be determined by either multiple tube fomentation technique (MPN method) or by membrane filtration technique (MF) procedure. The multiple tube fermentation technique, providing the most probable number (MPN) is an indirect count technique relaying on statistical interpretation of growth (gas formation) or no growth (no gas formation) observation in the inoculated tubes. The MPN test is conducted in three steps, namely presumptive test, confirmatory test and the completed test.

Generally, for routine examination, first step is sufficient. Water samples for this purpose were collected in properly cleaned and sterilized bottles. Microbiological analyses were carried out immediately after the collection of samples or else they were stored below 10°C, maximum for 6 hours during transit and in no case refrigeration storage time exceeded 30 hours.

Reagent

Lactose broth (McConkey) (Single and double strength) nutrient agar and bromocresol purple.

Procedure

A known volume of water sample and distilled sterile water were added to series of tubes containing McConkey broth of single and double strength. An indicator bromocresol purple was added to indicate colour change as a result of gas generation. An inverted Durham tube was placed in each tube to defect the presence of gas. The experimental design is summarized as bellow:

1) 5 and 10 ml each to 10 ml double strength lactose broth (DSLB).
2) 5 and 1 ml each to 5 ml single strength lactose broth (SSLB).
3) 5 and 0.1 each to 5 ml single strength lactose broth (SSIB).

From the number and distribution of positive/ negative reaction, the most probable of an indicator organism in the water sample as reported under McCrady’s probability standard table were calculated. This analysis is compared with (MPN) standard for drinking water. Accordingly, the quality of water, whether potable or non potable, was decided at each sampling station.

**Drinking Water Standards:**

In U.S.A. first public health service drinking water standards were applied to potable water in 1914. Subsequently, WHO has laid down International Standard for Drinking water in 1958. These have been revised in 1963 and 1971. India has made a special provision for the availability of safe drinking water for masses in VI five years plan. Accordingly, Bureau of Indian standards (IS:10:500 1991).

Number of counts was expressed as MPN/100 ml.10.

**Total plate count:**

Total plate count was done by incubating 0.1 ml diluted sample ($10^2$-$10^4$ times) in try tone-glucose agar medium at $35^0$C + $0.5^0$C for 38 hours. The number of colonies were counted and expressed as number of counts 100 ml sample.

**Escherichia Coliform (E. coli):**

The 0.1 ml diluted sample ($10^2$-$10^4$ times) was incubated in eosin methylene blue agar medium at $37^0$C±$1^0$C for 48 hours. There was development of pink to red colour with metallic surface sheet. This indicates the positive test for E. Coli.
Irrigation quality parameters:

The parameters for irrigation of water quality were also calculated with the help of some formulae given by (USDA, 1956, Goel, 1997, Mahida, 1981). For this purpose the result obtained after determination/estimation of Sodium (Na\(^{+}\)), Potassium (K\(^{+}\)), Calcium (Ca\(^{2+}\)), Magnesium (Mg\(^{2+}\)), Chloride (Cl\(^{-}\)), and Total alkalinity (as CaCO\(_3\)) in mg/l were converted in milliequivalent per liter (meq/l). These values of respected cation and anions were used in following calculations of respective parameter of irrigation quality for getting its index or ratios.

Sodium Absorption Ratio (SAR):

The index is used for predicting the sodium hazard of water in agricultural use. It is the concentration of sodium and proportion of sodium to calcium and magnesium. SAR was calculated as,

\[
\text{SAR (meq/l)} = \frac{\text{Na}^{+}}{\sqrt{\text{Ca}^{2+} + \text{Mg}^{2+}/2}}
\]

Residual Sodium Carbonate (RSC): If the water contains carbonate and bicarbonate in excess of calcium and magnesium then this excess is denoted as RSC and was calculated by following formula.

\[
\text{RSC (meq/l)} = (\text{HCO}_3^- - \text{CO}_3^-) - (\text{Ca}^{2+} + \text{Mg}^{2+})
\]

Kellys Ratio or Kellys Index (KR/KI):

It represent alkali hazard of water. KR is nothing but the proportion of sodium to calcium and magnesium and was calculated as,

\[
\text{KI (meq/l)} = \frac{\text{Na}^{2+}}{\text{Ca}^{2+} + \text{Mg}^{2+}}
\]
Soluble Sodium Percentage (SSP):

It is the percentage of sodium concentration to sodium, calcium and magnesium concentration and was calculated by following formula.

$$SSP \ (\text{meq/l}) = \frac{Na^+}{Ca^{2+} + Mg^{2+} + Na^+}$$

The result obtain for all above water analysis and calculation during the study period are presented in (Table 22 to 29).

Schollers Index (SCI):

This is the proportion of excess concentration of chloride to sodium and potassium to chloride and was calculated with following formula.

$$SSP \ (\text{meq/l}) = \frac{Cl^- (Na^+ K^+)}{Cl^-}$$

The result obtained for all above water analysis and calculation during study are presented in (Table number 21 to 29).

Soil:

In order to get an idea about development of salinity and or alkalinity as well as for variation in soil chemistry, the sampling stations were established along the length of stream and also across it. In all ten sampling locations were selected for the collection of soil samples.

Laboratory analysis of soil samples:

The collected soil samples were analyzed for determination of pH, EC exchangeable cations etc.
**EC and pH:**

For this, 1:5 soil: water suspension (in distilled water) was made and EC and pH of this suspension were determined with the help of digital EC and pH meter (Trivedy and Goel, 1986).

**Exchangeable Cations:**

In the laboratory, the exchangeable cations were determined by taking 25 gram of air dried soil and adding to it 100 ml of 40% alcohol and shaking the system. After fifteen minutes this suspension was filtered through ordinary filter paper and washed with 40% alcohol, twice. The scarped soil from the filter paper was taken in another beaker and 100 ml ammonium acetate was added to it and kept overnight. This suspension was filtered using an ordinary filter paper and washed it with ammonium acetate solution to make the final volume of 100 ml. Then, the filtrate was used in determination of the exchangeable cations viz. Ca$^{2+}$, Mg$^{2+}$, Na$^+$, and K$^+$ with the help of method described under water analysis with different formula which are given here as,

\[
\text{Ca}^{2+} \text{ in meq/100g} = \frac{A \times 400.8 \times V}{v \times 20.04 \times 10 \times S}
\]

\[
\text{Mg}^{2+} \text{ in meq/100g} = \frac{B-A \times 400.8 \times V}{v \times 1.645 \times 10 \times S \times 12.16}
\]

\[
\text{Na}^+ \text{ in meq/100g} = \frac{\text{mg Na}^+/ \text{ of soil extract} \times V}{10 \times S \times 23}
\]

\[
\text{K}^+ \text{ in meq/100g} = \frac{\text{mg K}^+ / \text{l of soil extract} \times v}{10 \times S \times 29}
\]

Where,

A= Volume of EDTA used for Ca$^{2+}$ determination in ml
B= Volume of EDTA used for Ca$^{2+}$ + Mg$^{2+}$ determination in ml.
V= Total volume of soil extract prepared.
S= Weight of soil taken.
V= Volume of extract titrated.

**Cation Exchange Capacity (CEC):**

It is the degree at which soil can absorb and exchange cations (positively charged ions). Hence, this test measures the soils ability to hold cation by electrical attraction. The CEC was calculated by following formula (Daji, 1985).

\[
CEC \text{ (meq/100g)} = \frac{\text{Na concentration of extract of soil} \times 100}{\text{Weight of soil in grams}}
\]

**Exchangeable Sodium Percentage (ESP):**

The ESP of the soil is the percentage of exchangeable sodium ions to the total exchangeable cations in the soil sample. It was calculated from the formula as given bellow (USDA, 1954).

\[
ESP \text{ (meq/100g)} = 100 \left( \frac{-0.0126 + 0.01475 \times SAR}{1 + (-0.126 + 0.01475 \times SAR)} \right)
\]

Similarly, the SAR and SSP for soil were also calculated as formulae described under water analysis.

The results obtained after analysis of soil are presented in (Tables 21 to 29). The correlation matrix was obtained within different properties of well water (Table 32) which is used to irrigate the soil (Table 31) in study area and soil and is presented in (Table 32). The elaborated and subsequent discussion on result of soil and water analysis is described in next chapters.