Impact of recharge sources on isotopic composition and microbiological quality of groundwater- a case study from Punjab, India

BY:

MS.SAROJ KUMARI

M.SC. (MICROBIOLOGY)

GKV HARIDWAR

UTTARAKHAND

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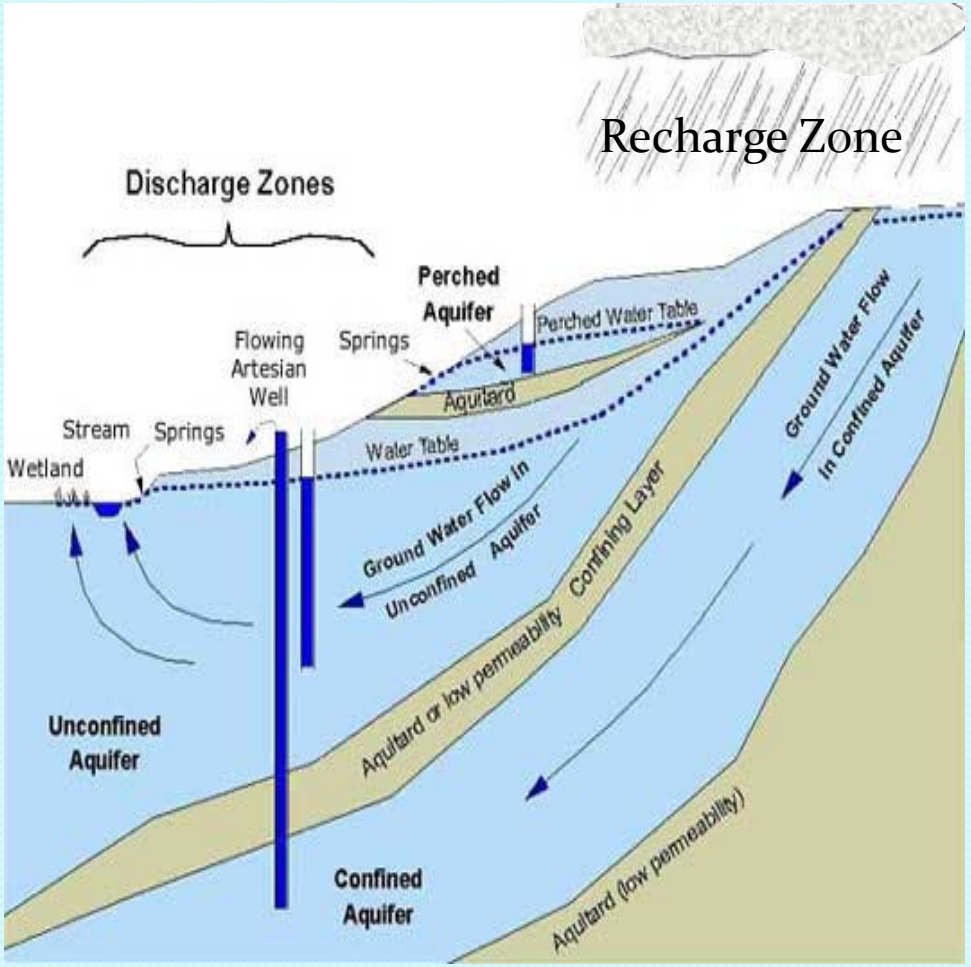
INTRODUCTION

- Groundwater is a vital element to sustain life and is widely used for drinking, irrigation and industrial purposes
- Groundwater is found in aquifers, which are the layers below the ground with the capability of both storing and transmitting the water.
- There was little concern with groundwater contamination
- Microbial contamination of groundwater is increasing due to an increase in number of point and nonpoint sources of pollution

Importance of groundwater

- Filtered out by the natural process of filtration.
- Free from pathogenic bacteria
- Omni present
- Contains the required minerals



Schematic flow of aquifer in groundwater

AIM & OBJECTIVES:-

The objectives of present study "**Impact of recharge sources on isotopic composition and microbiological quality of groundwater were:**

- To investigate the microbiological quality and isotopic tracing of groundwater flow paths .

- To investigate the source of recharge and flow conditions of groundwater by isotopic analysis.

There are majority of bacteria are found in water belong to groups:

- Fluorescent bacteria (eg. *Pseudomonas, Alginomonas*, etc.) coliform group (*E.coli, Aerobactor*, etc.).

- *Proteus* group, non-gas forming, non-chromogenic and non–spore forming rods, spore former of the genus Bacillus.

- Pigmented and non- pigmented cocci (*Micrococcus)*

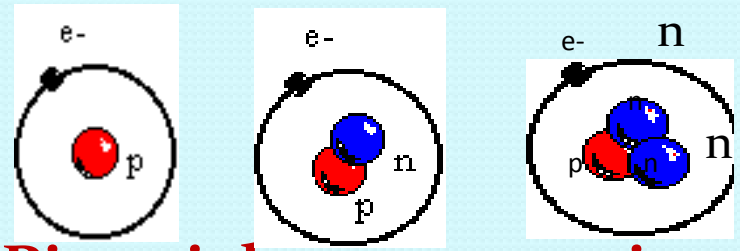
ISOTOPES

The isotopes are the atoms with same atomic no.(p) but different atomic mass no. (n). For example, hydrogen has three isotopes having the same atomic no.(p=1) but different atomic mass no.(n=0,1,2).

$^{1}_{1}H$ - 1proton in nucleus,

$^{2}_{1}H$ - 1 proton + 1 neutron and

$^{3}_{1}H$ - one proton+2 neutrons.



Pictorial representation of isotopes of hydrogen

$^{1}_{1}H$ $^{2}_{1}H$ $^{3}_{1}H$

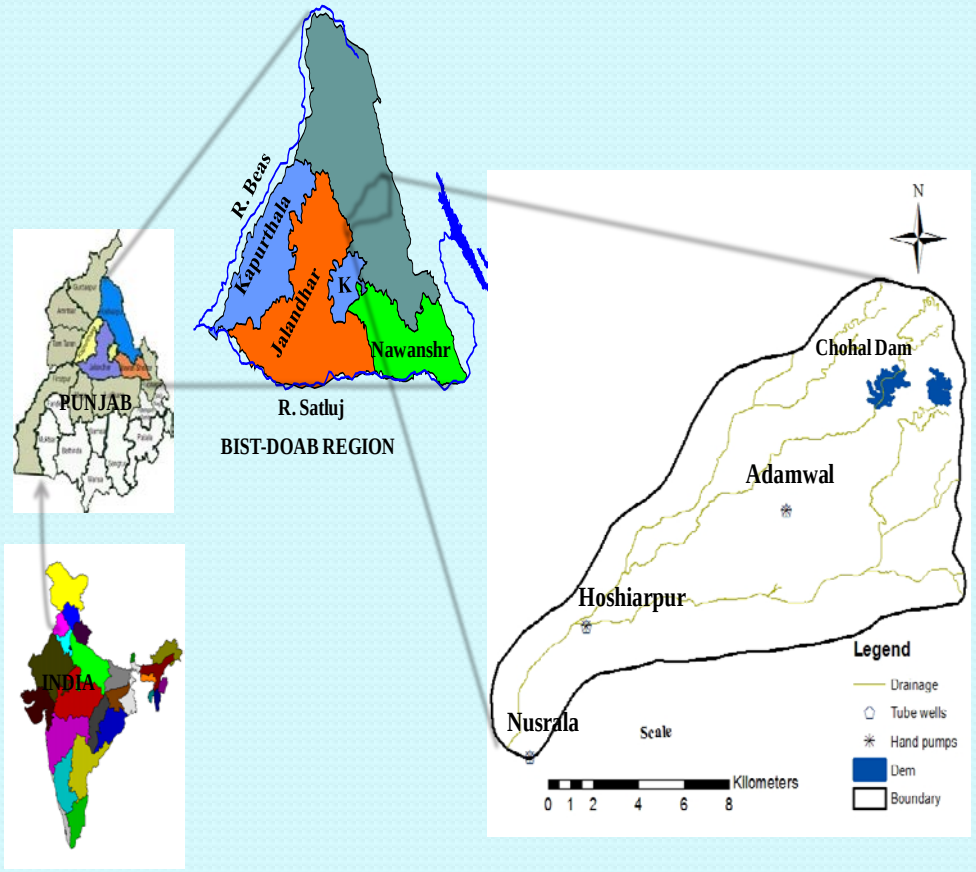
Hydrogen Deuterium Tritium

Water (H_2O) from different origin can be traced using isotope analysis

REVIEW OF LITERATURE

- For more than 100 years, the microbial safety of drinking water has primarily been determined by testing for bacterial 'indicators' of faecal pollution, mainly *Escherichia coli* (*E coli*)
- Bacteria existed down to 1240 m below ground in a borehole in mine with colonies 4 x 10^3 to 8 x 10^5 more attached than unattached bacteria in a fracture channel and that the attached bacteria were more active than the unattached bacteria **(Pedersen & Ekendahl, 1992).**
- Pollution of surface flow and ground water from application of animal waste has been well documented **(Mallin et al., 1997; Mawdsley et al., 1995)** Liquid –waste discharge onto soil initiates solute and microbe movement that follows natural ground water drainage patterns and may contaminate ground water

STUDY AREA



Study area and samples location

Present study is taken up in the Hoshiarpur district located in the Beas-Satluj Doab region of the Punjab state. Hoshiarpur district falls in the eastern part of the Punjab State .

Latitudes 30°58'30"N and 32°08'00'N
Longitudes 75°28'00" E and 76°30'00"E
The population density of the district is 440 persons per sq.km

Generally rainfall in the district increases from southwest to northeast.

- Normal Annual Rainfall : 938 mm
- Normal monsoon Rainfall : 720 mm
- Normal Rainy days : 38
- Mean Maximum Temperature : 39°C (May June)
- Mean Minimum Temperature : 5°C (January)

The study of exploratory boreholes drilled by of Central Ground Water Board indicated presence of three aquifer group's up to 425m depth below ground level.

MATERIALS AND METHODS

(A) Microbiological Analysis
(B) Isotopic Analysis

Microbiological Analysis

Materials

- Bunsen burner
- Laminar air flow
- Microscope
- Oven
- Incubator
- Autoclave
- Hot plate
- Quebec colony counter
- Balances
- Refrigerator

METHODS

Steps included in the isolation and identification of the bacteria in water sample

- Isolation of bacteria by serial dilution agar plate method
- Medium preparation for the growth of the bacteria.
- Calculation of the total no. of bacteria in per ml of the water sample.
- Isolation of discrete colonies from a mixed culture by streak plate method.
- Identification of isolated bacterial culture by Gram's staining and Biochemical characterization

Serial dilution agar plate method

- Prepared the seven blanks in sterilized test tubes with distilled water covered by cotton plugs. Labeled the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}.
- Shake the water sample vigoursaly to obtain the uniform distribution of organisms.
- From the first dilution, transferred 1ml of the suspension to the dilution blank 10^{-2} with sterile and fresh 1ml pipette and repeated the process up to the dilution blank 10^{-6}
- Transferred 1ml of suspension while in motion with the respective pipette to sterile Petri plate. Three Petri plates were used for each dilution.
- Added approximately 15ml of melted nutrient agar medium. To each Petri plate containing diluted sample.
- Allowed the medium to solidify in plates.
- Incubated the Petri plates in an inverted position for 24-48 hrs. at 37°c

CALCULATION

The bacterial concentration is calculated using the following formula

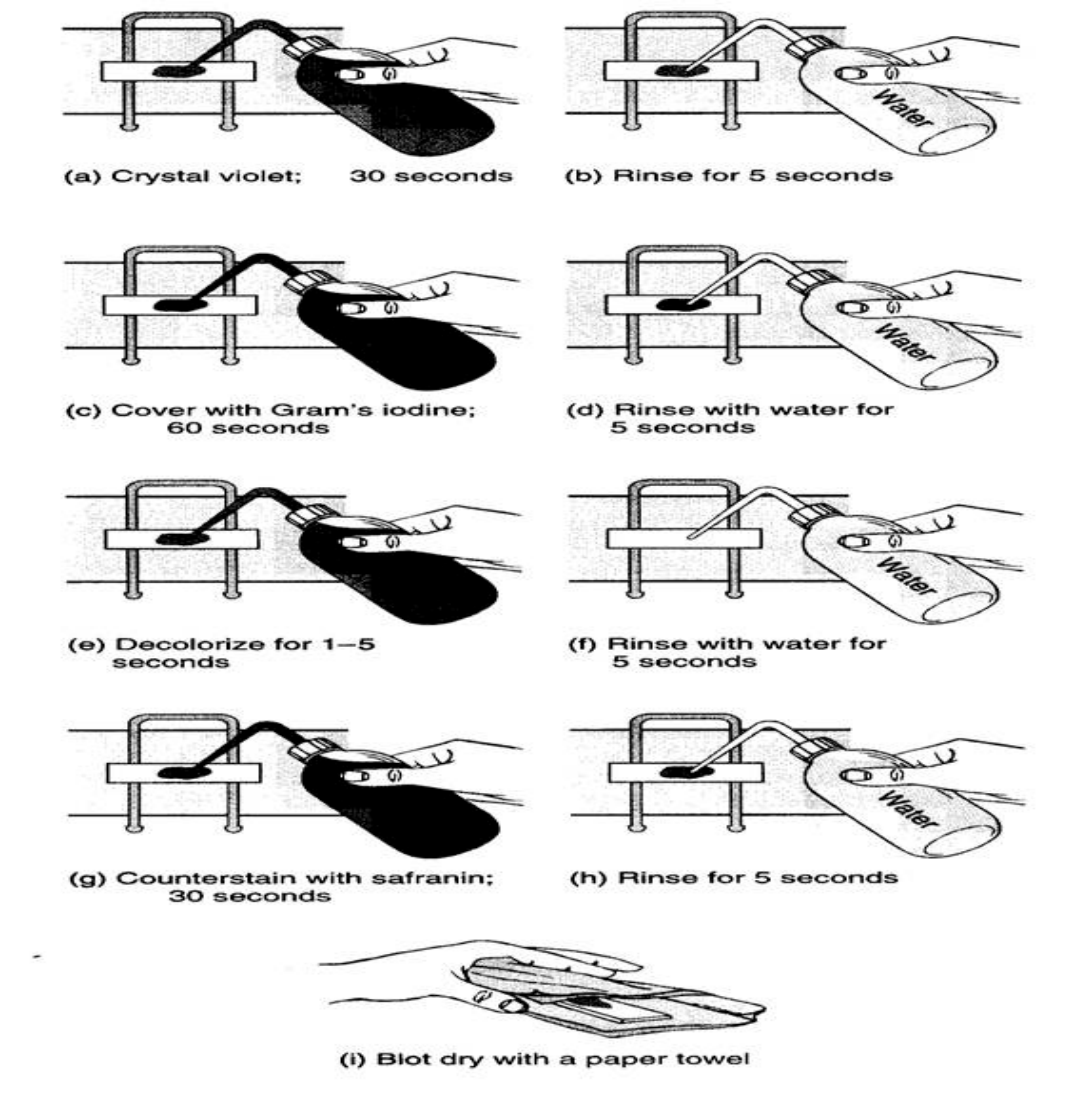
$$\text{No. of cells / ml or g} = \frac{\text{No. of colonies x dilution factor}}{\text{Quantity of water samples}}$$

(In the present study, 1 ml of water sample is taken for the analysis)

Isolation of pure culture by using streak plate method

- Poured the melted nutrient agar medium in the sterilized Petri plates.
- Allowed the medium to solidify
- Sterilized the wire loop.
- Placed the loop on the bacterial colony in the mix culture and pick the particular colony.
- Dragged the loop over the surface of the top one-third of the plate back and forth in a "zigzag" formation.
- The loop has picked up thousands of bacteria which are spread out over the surface of the agar.
- Sterilized the loop in the flame.
- Turned the plate 90 degrees and drag the loop through the area which have just streaked and continue to drag the loop in a "zigzag" manner without touching that area again.
- Sterilized the loop in the flame.
- Incubated the plate for 24-48 hours in the incubator at 37°c

Identification of isolated bacterial culture (by Gram's staining and Biochemical tests)



Procedure of Gram's staining method

BIOCHEMICALS TESTS

Catalase Activity test

Hydrogen peroxide was added into the slants drop by drop and the release of bubble was noted

Starch hydrolysis test

- Melted the starch medium. Cool and pour into sterile Petri plates. And allowed it to solidify.
- Labeled each plate with the name of organism to be inoculated.
- Make a single streak inoculation of organism into the centre of its appropriately labeled plate in the Zigzag manner.
- Incubated for 24-48 hrs at 37° c in the inverted position.
- Flooded the surface with the iodine solution with dropper for 30 sec.
- Poured off excess iodine solution.

ISOTOPIC ANALYSIS OF WATER

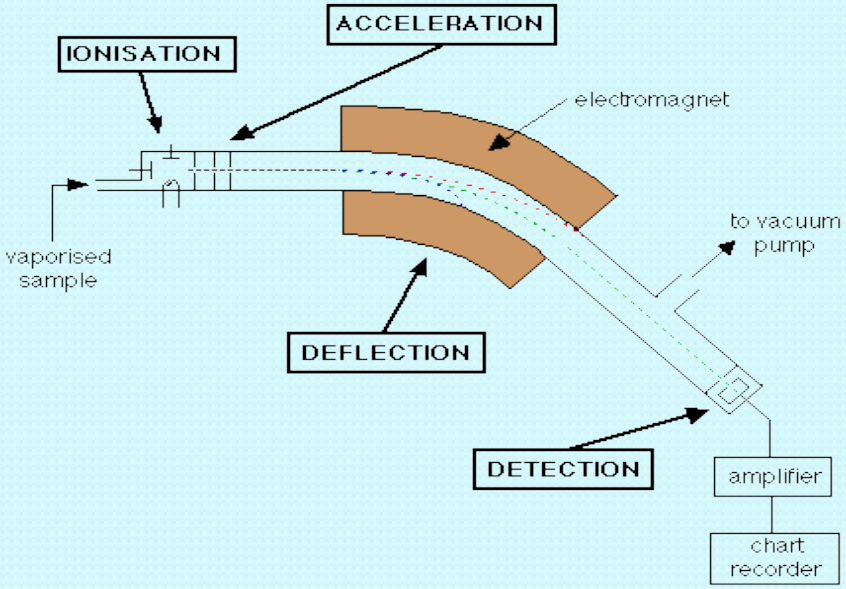
The isotopes and their abundance in sample are measured on mass spectrometer. The measurement of absolute abundance of isotope of an element requires dedicated mass spectrometer. To avoid this problem, generally, rather than measuring the absolute values relative abundance of rare isotope with respect to the most abundant isotope of the same element is measured. The ratio is termed as isotope ratio. The symbol ‰ (per mill) is per thousand similar to % as per hundred. The ratio is expressed in 'delta' (δ). For example, δ ^{2}H for hydrogen is expressed as;

$$\delta^2H = \frac{\left(\frac{^2H}{^1H}\right)_{sample} - \left(\frac{^2H}{^1H}\right)_{std}}{\left(\frac{^2H}{^1H}\right)_{std}} \times 1000\ ‰$$

Similarly, $\delta^{18}O$, isotope ratio for oxygen is defined as;

$$\delta^{18}O = \frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample} - \left(\frac{^{18}O}{^{16}O}\right)_{Std}}{\left(\frac{^{18}O}{^{16}O}\right)_{Std}} \times 1000\ \%$$

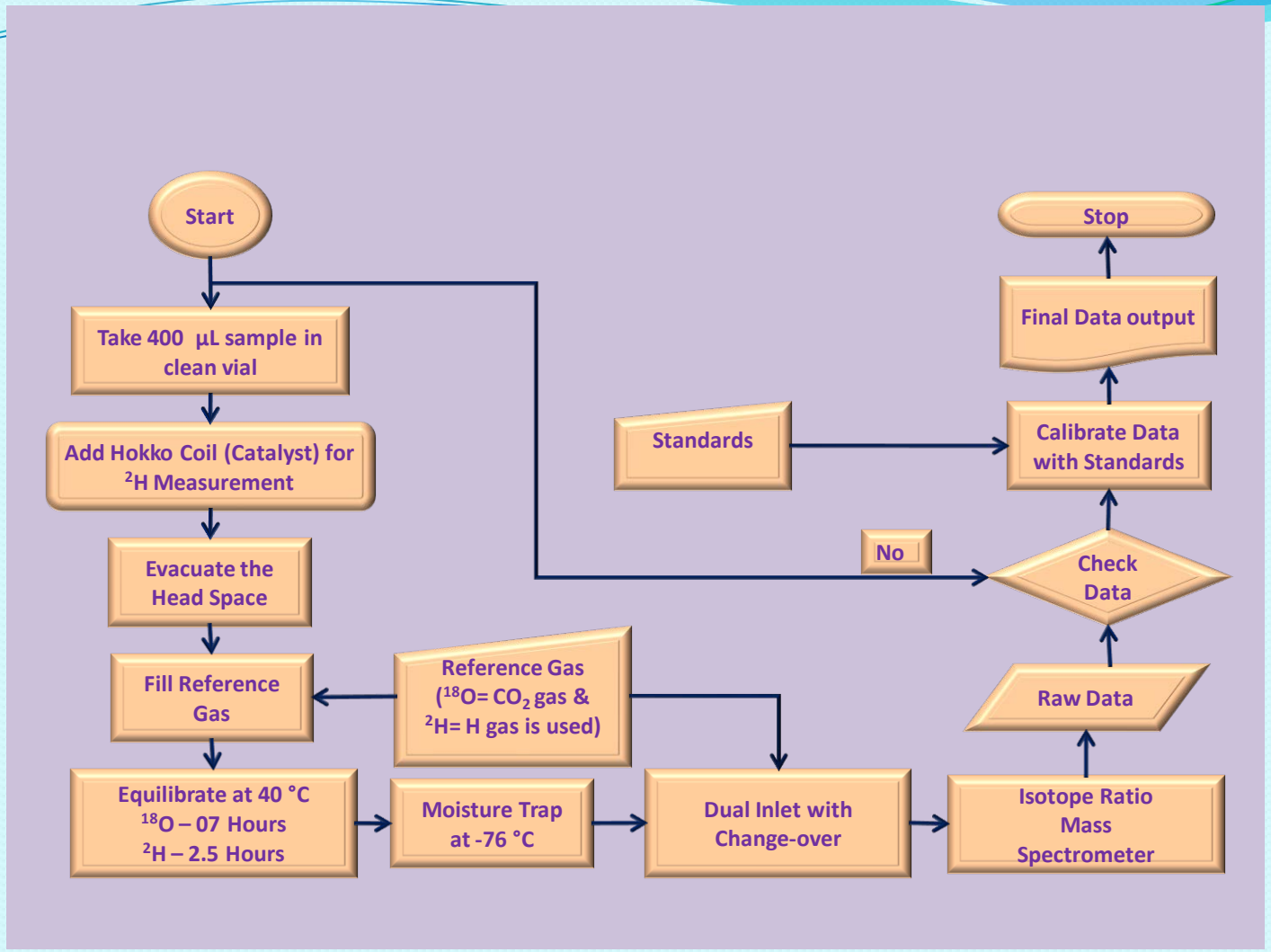
Continous flow-Isotope ratio mass spectrometer system at the National Institute of Hydrology, Roorkee



Isotope analysis in a mass spectrometer

A mass spectrometer ionizes the gas molecules and separates the ions into a spectrum according to charge- to- mass ratio, m/z, using electric and magnetic fields. The relative abundance of molecules of different m/z are then measured from the currents generated by these separated ion beams. All the instruments have three basics parts: an ion source, a mass analyzer and an ion collector assembly

Isotopes are useful in tracing the groundwater flow paths and in analyzing the mixing ratio quantitatively for multiple recharging sources forming the groundwater. Isotope ratio provides information on the rate of chemical reaction, evaporation effects, condensation process, diffusive processes etc. Similar to DNA fingerprinting, Isotope provides fingerprint indexing to the recharge sources.



Flow chart for the Analysis of Stable Isotopes (δ^{18} & $\delta^{2}H$) on Dual Inlet Isotope Ratio Mass Spectrometer

RESULTS

Microbiological

- Bacterial conc. is higher in deep region as compared to the shallow region.
- In some regions same type of the bacteria is found in both shallow and deep region which show common characteristics in the climate of that place.

(A) Microbiological

Enumeration of bacterial population from Adamwal region

Dilutions	shallow region (average no. of colony)	deep region (average no. of colony)
10^{-4}	65×10^4	110×10^4
10^{-5}	31×10^5	57×10^5
10^{-6}	24×10^6	44×10^6

* in average triplicates

Enumeration of bacterial population from Hoshiarpur region

Dilutions	shallow region (average no. of colony)	deep region(average no. of colony)
10^{-4}	63×10^{4}	102×10^{4}
10^{-5}	35×10^{5}	95×10^{5}
10^{-6}	35×10^{6}	91×10^{6}

* in average triplicates

Enumeration of bacterial population from Nusrala region

Dilutions	shallow region (average no. of colony)	deep region (average no. of colony)
10^{-4}	76×10^{4}	110×10^{4}
10^{-5}	30×10^{5}	65×10^{5}
10^{-6}	26×10^{6}	30×10^{6}

* in average triplicates

Morphological and biochemical characterization of bacteria from Adamwal region

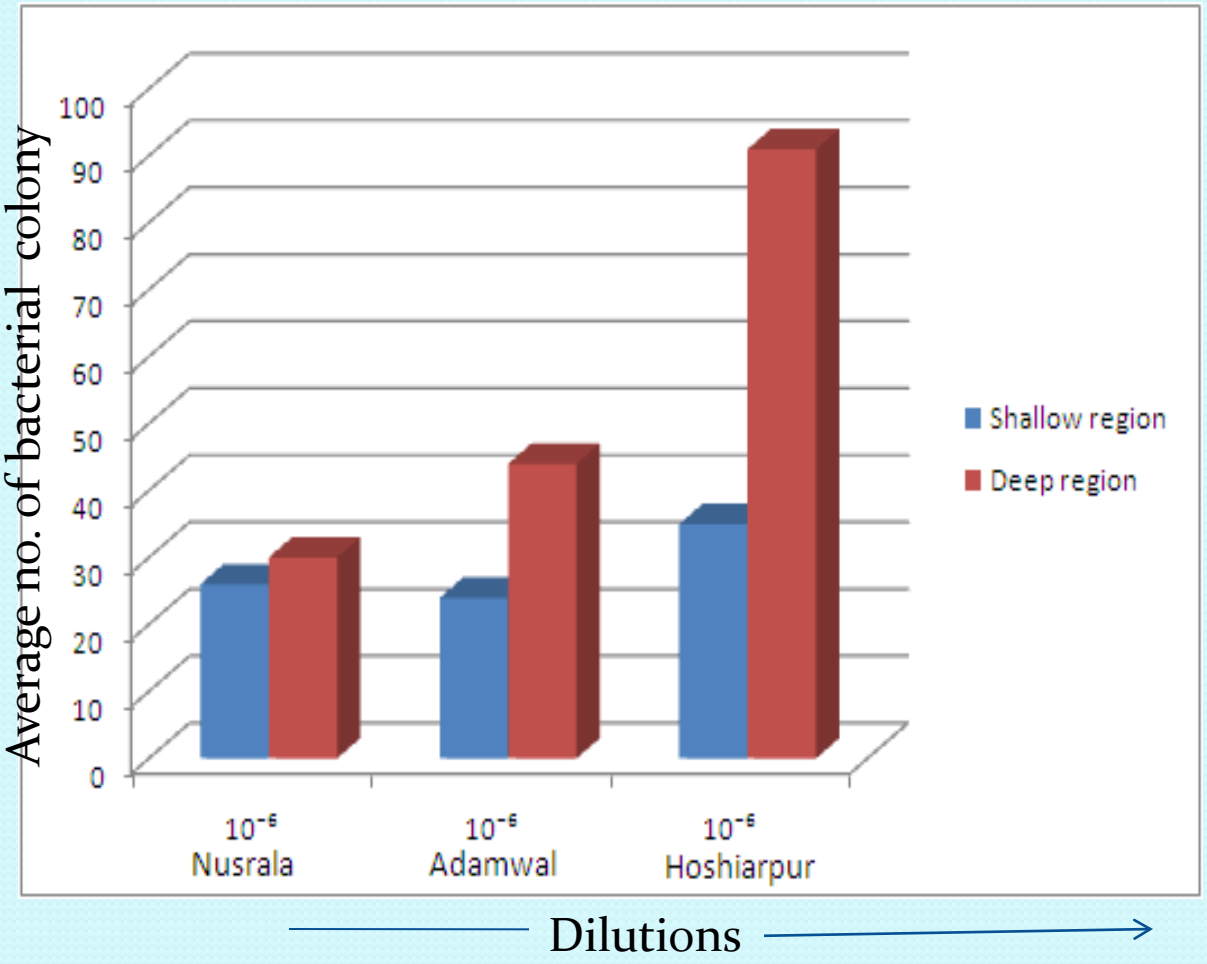
CHARACTERSTICS	**SHALLOW REGION**	**DEEP REGION**	
Morphological	***Staphylococcus aureus***	***Staphylococcus aureus***	***Streptococcus lactis***
Growth	moderate	Moderate	moderate
Form	Circular	Circular	Circular
Margins	Entire	Entire	Entire
Pigmentation	white	White	Yellow
Biochemical tests			
Gram staining	-ve rods	-ve rods	+ve coccus
Catalase test	+	+	-
Starch hydrolysis	-	-	-

Morphological and biochemical characterization of bacteria from Hoshiarpur region

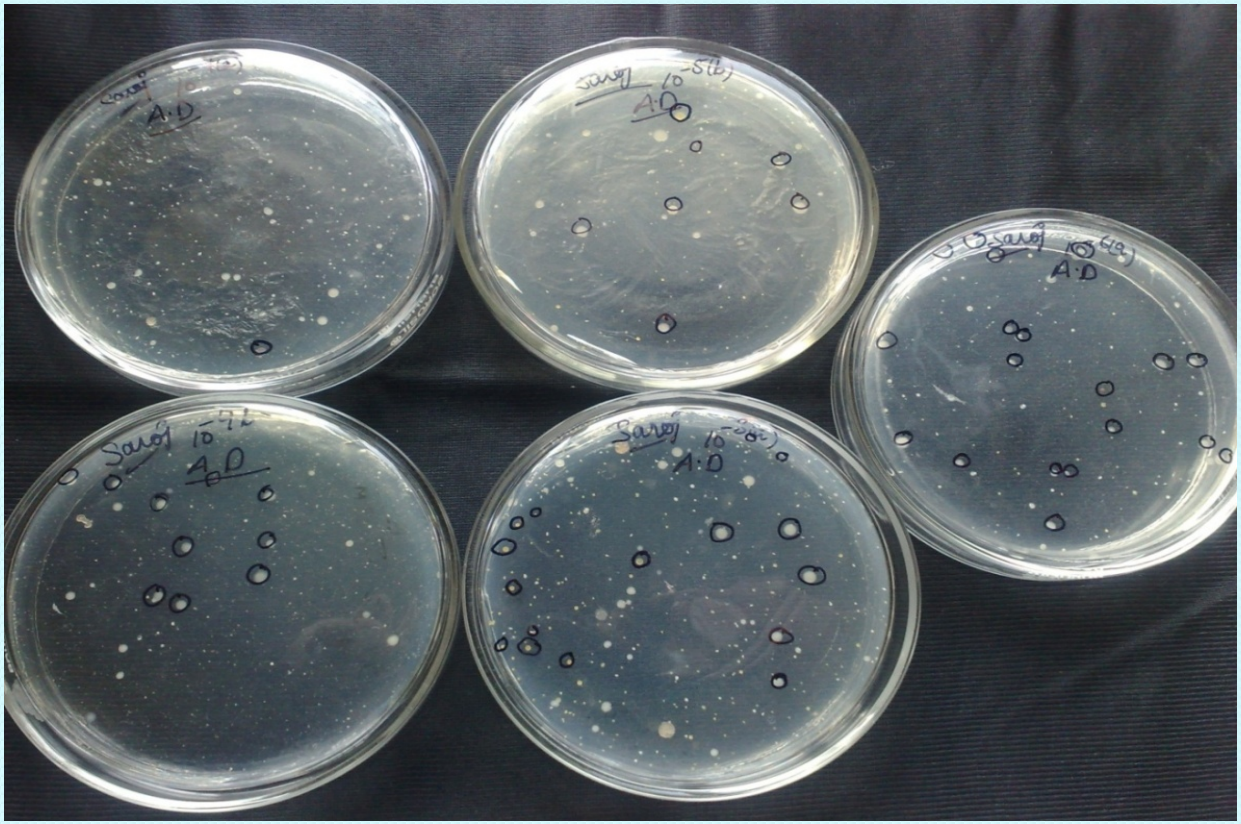
CHARACTERSTICS	SHALLOW REGION		DEEP REGION
Morphological	***Bacillus sp.***	***E.coli***	***E.coli***
Growth	Slight, Waxy growth	Moderate	Moderate
Form	Circular	Circular	Circular
Margins	Entire	Entire	Entire
Pigmentation	White	Yellow	Yellow
Biochemical tests			
Gram staining	+ve rods	-ve rods	-ve rods
Catalase test	+	+	+
Starch hydrolysis test	+	-	-

Morphological and biochemical characterization of bacteria from Nusrala region

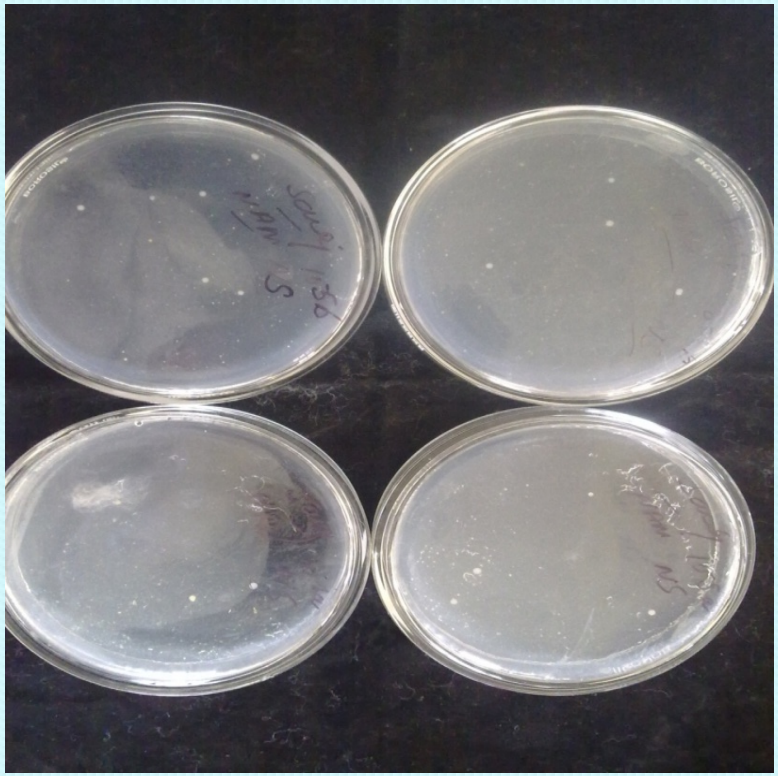
CHARACTERSTICS	**SHALLOW REGION**		**DEEP REGION**	
Morphological characteristics	***Streptococcus lactis***	***Micrococcus***	***E.coli***	***Bacillus***
Growth	moderate	Abundant	Moderate	Slight, waxy growth
Form	Circular	Circular	Circular	circular
Margins	Entire	Entire	Entire	Entire
Pigmentation	Yellow	Creamish	Yellow	White
Biochemical tests				
Gram staining	+ve coccus		-ve rods	+ve rods
Catalase test	-		+	+
Starch hydrolysis test	-		-	+

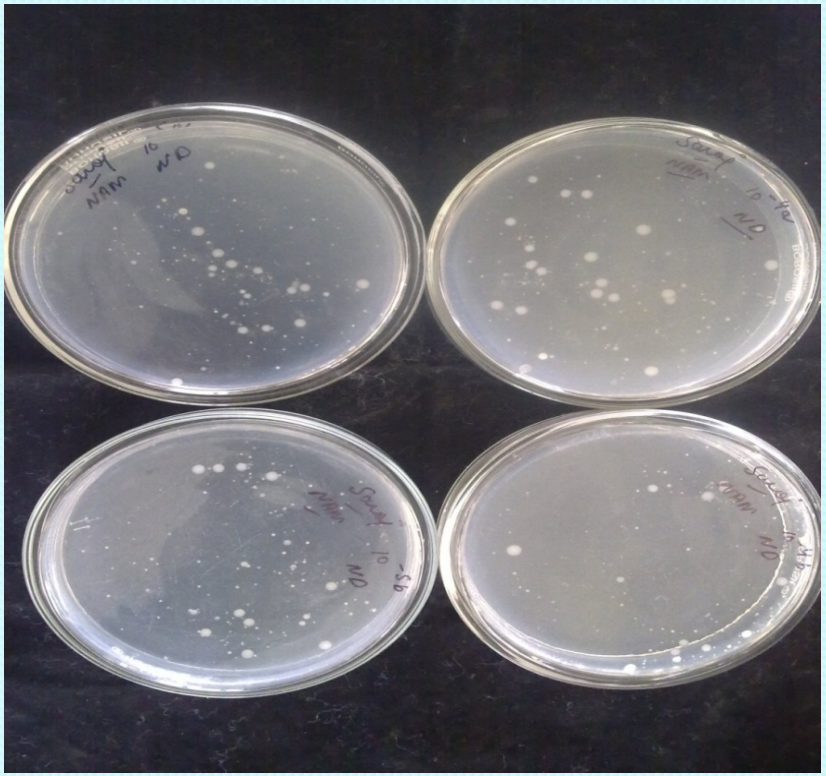


3D diagram show the bacterial population in Nusrala, Adamwal and Hoshiarpur



Bacterial colonies on the different dilutions of the Deep region of Adamwal.





Shallow region

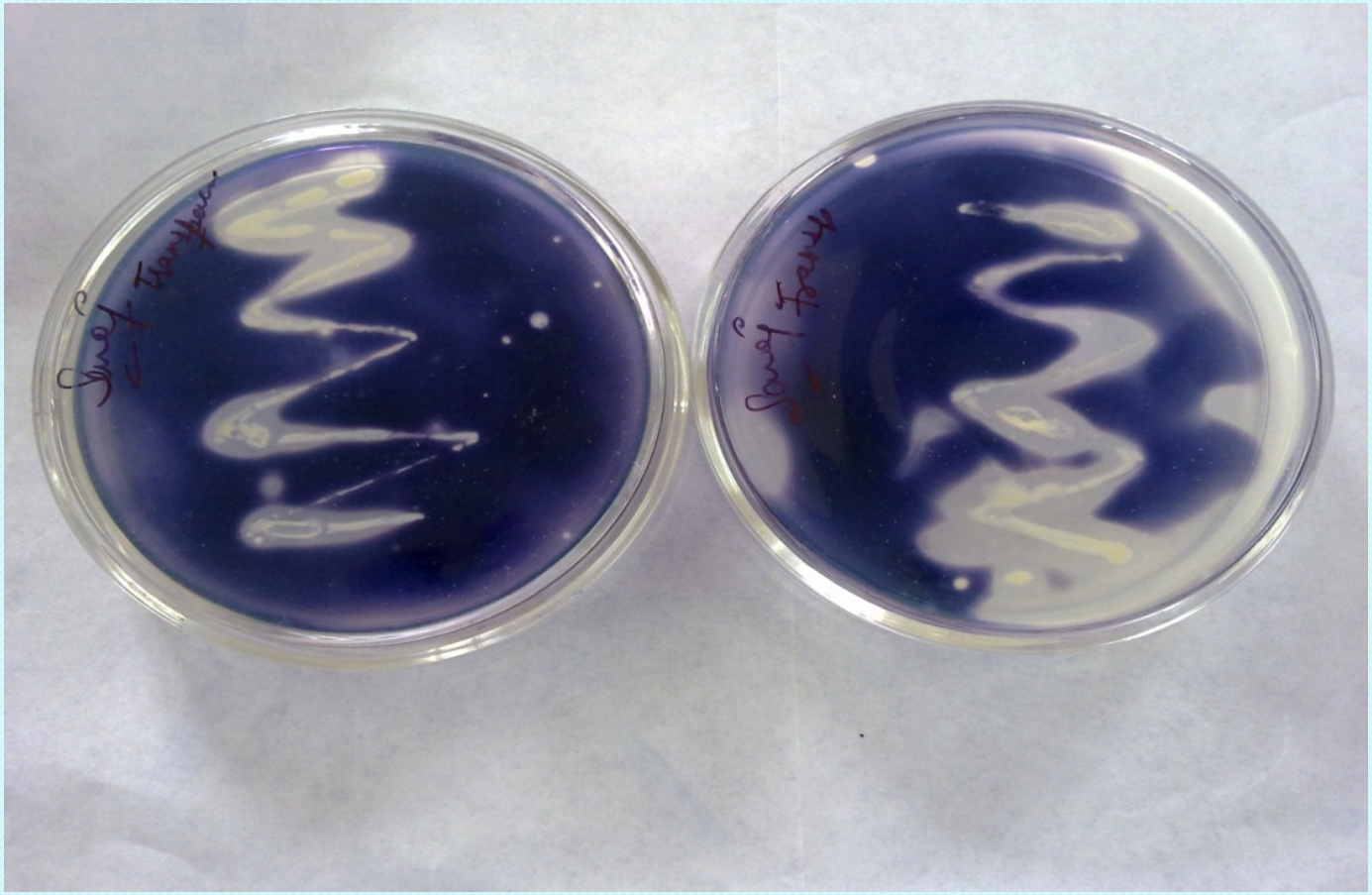
Deep region

Bacterial colonies in different depth of the Nusrala region

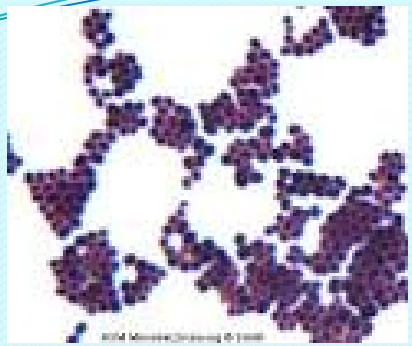
Pure culture of the *E.coli*

Pure culture of *Bacillus sp.*

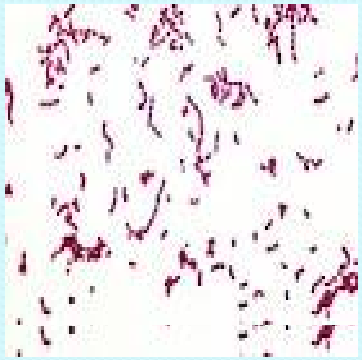
Pure culture of *Micrococcus*



Starch Hydrolysis test of identified bacterial species



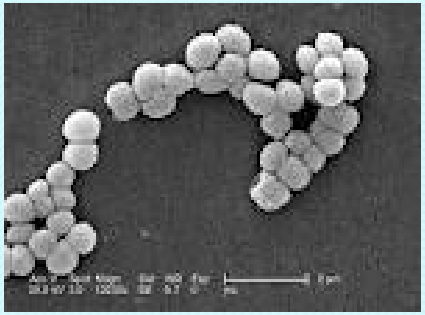
Staphylococcus aureus



Streptococcus lactis

E.coli

Bacillus sp.



Micrococcus luteus

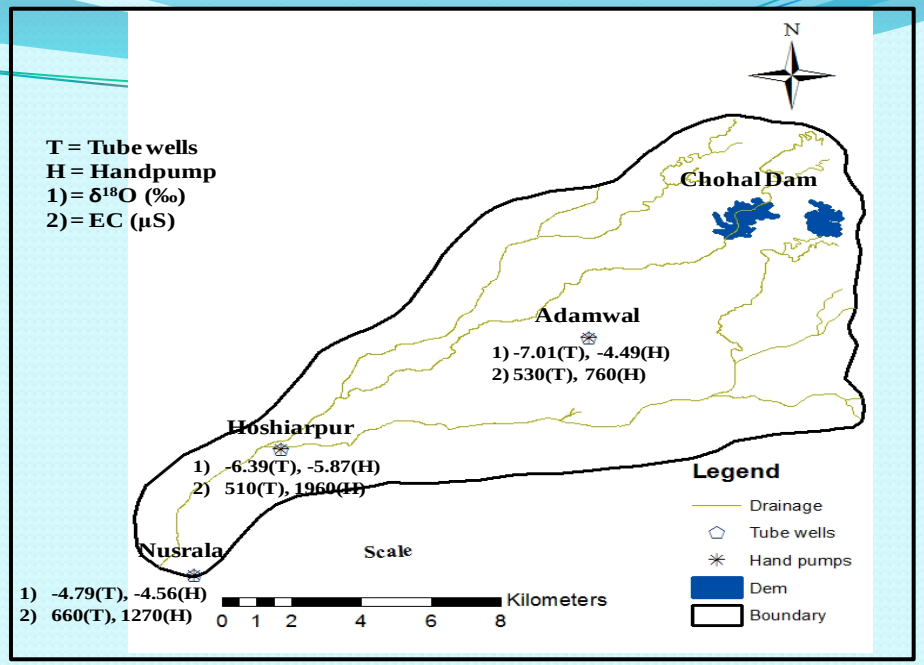
Microscopic view of identified bacterial species

Isotopic results of collected water samples

S.No.	Sample location	Depths (m)	Altitude (m)	EC (µs/cm)	$\delta^{18}O$	δD
1	Adamwal	24.38	323	760	-4.49	-37.98
2	Adamwal	228.6		530	-7.01	-47.36
3	Hoshiarpur	30.48	313	1960	-5.87	-39.57
4	Hoshiarpur	231.64		510	-6.39	-43.24
5	Nusrala	18.288	267	1270	-4.56	-35.24
6	Nusrala	137.16		660	-4.79	-38.50

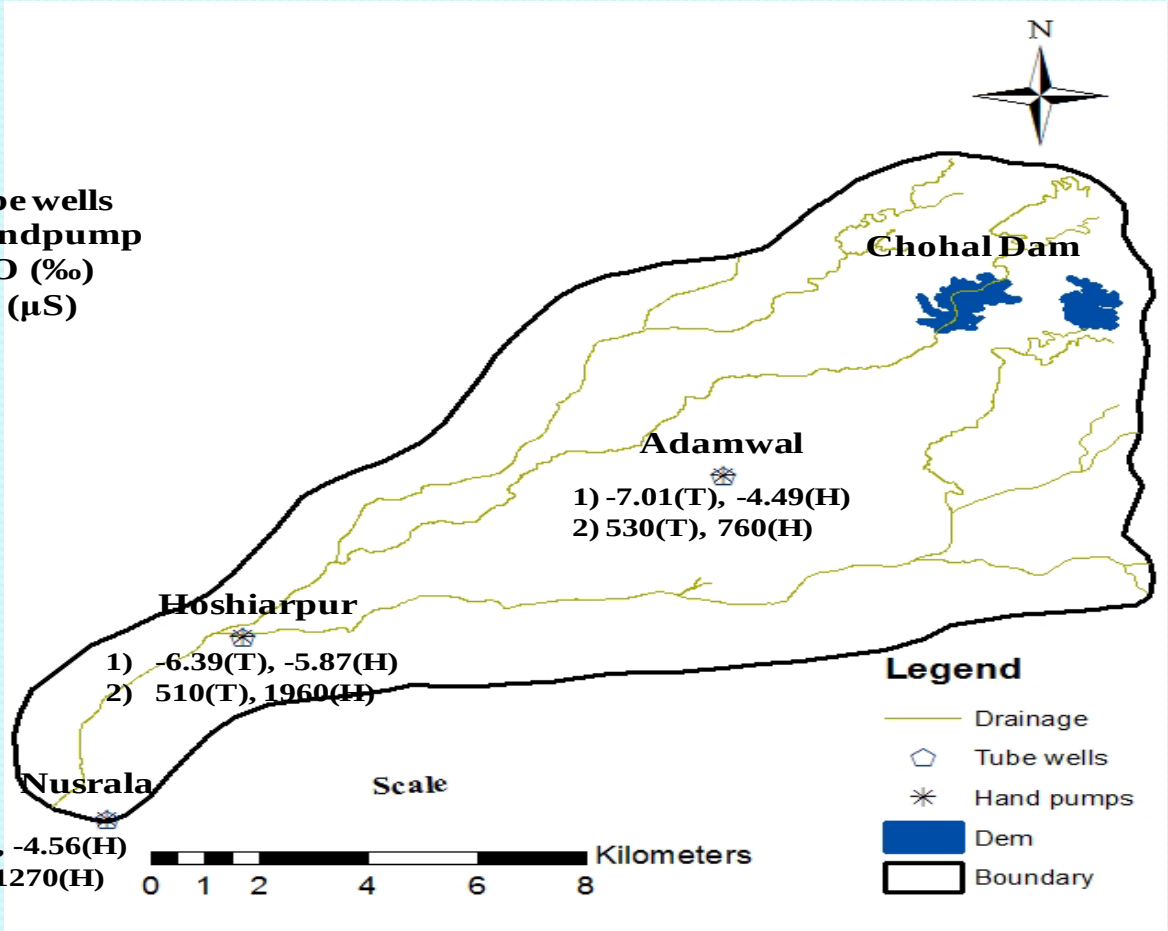
Isotopic

In Adamwal groundwater in deep aquifer is more depleted (-7.01) with respect to the isotopic composition of the shallow water(-4.49) indicates the recharge of deep groundwater from higher altitude.

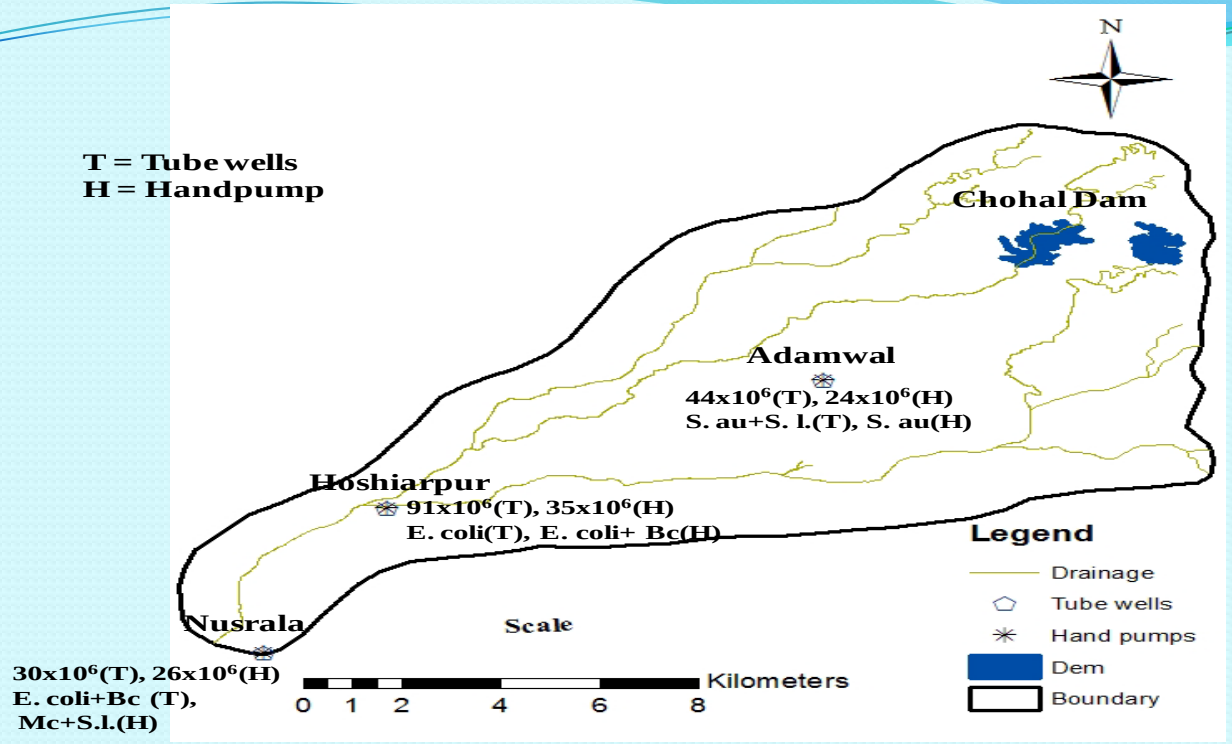


At Hoshiarpur (-4.49 to -5.87) ,the isotopic difference between shallow and deep groundwater get reduced.

- At Nusrala both shallow and deep aquifer shows enriched isotopic compositionment.
- Groundwater salinity in deep aquifer is low at all sites as compared to shallow region, which indicates anthropogenic source of contamination.
- Salinity wise shallow and deep groundwater in Adamwal is fresh.



Stable isotopic composition and salinity (EC) of groundwater in the study area



Bacteria type and population distribution in groundwater in the study area. (Abbreviations used: S. au: *Staphylococcus aureus*; S.l.: *Streptococcus lactis*; Mc: *Micrococcus luteus* and Bc:*Bacillus spp*.)

CONCLUSION

- Bacteriological colonies can infiltrate and contaminate even deep aquifer wherever there is interaction between shallow and deep groundwater.
- Using isotopes groundwater recharge source and interaction between shallow and deep aquifer can be monitored.
- Pollution due to antheropogenical influence change the salinity of groundwater.
- Natural fresh water recharge freshen the quality of groundwater.
- The extent of freshening depends upon the level of the pre-contamination and fresh water recharge conditions.

THANKS