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RESEARCH ARTICLE

TO STUDY THE PHYSICO-CHEMICAL PROPERTIES AND BACTERIOLOGICAL EXAMINATION OF STEPWELL WATER FROM MAJHWAR REGION IN DISTT. MANDI OF HIMACHAL PRADESH, INDIA

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ABSTRACT

Water is basic and vital resource on earth. Due to increase in population, industrialization and pollution, water has became polluted. In this study, physico-chemical properties and bacteriological examination of stepwell water from Majhwar region in Distt. Mandi of Himachal Pradesh, India was done. Among the physico-chemical analysis, Total Dissolved Soilds (TDS), Disssolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Alkalinity, Hardness, Chloride and pH were checked. Bacteriological analysis was made by multiple tube fermentation test which include: Presumptive coliform test, confirmed coliform test and completed coliform test. All the physico-chemical parameters were compared with WHO standard and it was found that water was potable.

INTRODUCTION

Stepwells, also called Kalyani or Pushkarani. Stepwell are used for water storage and irrigation in India and cope seasonal fluctuations in water availability. Stepwells usually consist of two parts: a vertical shaft from which water is drawn and the surrounding inclined subterranean passageways, chambers and steps which provide access to the well. (Davies, 1989). Stepwell construction is known to have gone on from at least 600 AD in the south western region of the first rock-cut step wells in India date from 200-400 AD (Livingston, 2002). Fresh water is one of the most important resource for the survival of all the living beings. It is also important for the human being as they depend upon it for food production, industrial and waste disposal (Agarwal *et al*., 1982). As of now only earth is the planet having about 70 % of water. But due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants. Therefore it is necessary that the quality of drinking water should be checked at regular time interval, because due to use of contaminated drinking water, human population suffers from varied of water borne diseases. (Basavaraja *et al.,* 2011). Natural water contains different types of impurities are introduced in to aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities, including mining, processing and the use of metal based materials (Ipinmoroti and Oshodi, 1993; Adeyeye, 1994; Asaolu *et al*., 1997). Water causes water born disease which has led to the death of millions of people. (Adefemi and Awokunmi, 2010). People on globe are under tremendous threat due to undesired changes in the physical, chemical and biological characteristics of air, water and soil. These are related to animal and plants and finally affecting on it (Misra and Dinesh, 1991). Development of industries results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution (Fakayode and Onianwa, 2002; Fakayode, 2005). Pollution parameters have been classified as physical, chemical and biological on the basis of analytical tests. Physical parameters include temperature, turbidity, colour, odour, taste, suspended and floating matter etc. manifesting palatability and aestheticity. Chemical parameters include organic and inorganic Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nitrogen in various forms, pH, alkalinity, chlorides, sulphates, heavy metals, pesticides etc. Biological parameters include coliform bacteria, pathogens; nuisance organisms total plate count, bioassay, species diversity etc. (Kumar *et al*., 2012). Physico-Chemical Properties of water such as Total Dissolved Soilds (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Alkalinity, Hardness, Chloride and pH from

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Markanday Spring in Hamirpur District of Himachal Pradesh were also reported (Kumar and Nath, 2013). Present study was done to study the physico-chemical properties and bacteriological examination of stepwell water from Majhwar region in Distt. Mandi of Himachal Pradesh. Majhwar is a village which is located 10 km away from Mandi. It is located approximately along 31. 6867 latitude and 76.9697 longitude.

MATERIALS AND METHODS

Water sample was collected in sterile bottles from stepwell of Majwar region on 2nd March, 2013 and brought to laboratory at Abhilashi Institute of Life Science (AILS), Tanda, Mandi.

A. Physico-chemical analysis: Following physico-chemical analysis were studied:

1. Total Dissolved Solids (T.D.S.) of water (Aneja, 2003)

Took the weight of the evaporating dish. Filtered the 50 ml of water sample through Whatman filter paper no.4. Transferred the water sample to the evaporating dish. Evaporate on a water bath. Noted the weight of the dish along with the contents after cooling in desiccators. Total Dissolved Solid was calculated as per formula:

$$\text{T.D.S of water} = \frac{(B-A)}{V} \times 10^6$$

Where B= Final weight of beaker
A= Initial weight of beaker
V= Volume of sample

2. Determination of Dissolved Oxygen (DO) of water ((Aneja, 2003)

Took the water sample in the 250 ml bottle. Added 2 ml each of manganous sulphate and alkaline potassium iodide solution in succession right at the bottom of the bottle with separate pipette and replaced the stopper. Shook the bottle in the upside down direction at least six times. Allowed the brown ppt. to settle. Added 2 ml of conc. Sulfuric acid and shook the stoppered bottle to dissolved the brown ppt. Took 50 ml sample in a flask and titrated with thiosulphate solution till the colour changed to pale straw. Added 2 drops of starch solution to the above flask which changed the colour of the contents from pale to blue. Titrated again with thiosulphate solution till the blue colour disappeared. Dissolved Oxygen (D.O.) was calculated as per:

$$\text{DO of water sample (mg/lt)} = \frac{8\times1000\times N\times v}{V}$$

Where N = Normality of solvent
V = Volume of water sample
v = volume of titrant used
8 = Constant

3. Determination of Biochemical Oxygen Demand (B.O.D.) of water ((Aneja, 2003)

Adjusted the pH of the water sample to neutrality 1N acid or 1N alkali solution. Filled the water sample in 6 BOD bottles without bubbling. Added 1 ml of allylthiourea to each bottle. Determined dissolved oxygen content in three of the 6 BOD bottle by the titration method. Took the mean of the three readings (D1). Incubated the rest of the three BOD bottle at 27°C in a BOD incubator for 3 days. Estimated the oxygen concentration in all the 3 incubated samples. Took the mean of the three readings (D2). Calculated Biochemical Oxygen Demand (B.O.D.) of water as per:

B.O.D. = D2 – D1

4. Determination of the Chemical Oxygen Demand (C.O.D.) of water ((Aneja, 2003)

Took three 100 ml conical flasks and poured 50 ml water sample in each. Simultaneously run distilled water blanks standards. Added 5 ml of potassium dichromate in each of the six flasks. Kept the flasks in water bath at 100°C for one hour. Allowed the sample to cool for 10 minutes. Added 5 ml of potassium iodide in each flask. Added 10 ml of sulfuric acid in each flask. Titrated the content of each flask with 0.025 N sodium thiosulphate until the appearance of the pale yellow colour. Added 1 ml of starch solution to each flask. Titrated it against with 0.025N sodium thiosulphate until the blue colour disappears completely. Chemical Oxygen Demand (C.O.D.) of water was calculated as per formula:

$$\text{COD of water sample} = \frac{8\times N\times(B-A)}{V}$$

Where N = Normality of solvent
B = Mean value of titrant for blank B
A = Mean value of titrant for blank A
V = Volume of water taken
8 = Constant

5. Determination of Alkalinity of water

Pipetted 50 ml of water sample into a clean flask. Added one drop of sodium thiosulphate solution if residue of chlorine is present. Added two drops of phenolphthalein indicator if the pH is above 8.3 colour of sodium become pink. Titrated against standard solution sulfuric acid in the burette till the colour just disappears. Noted down the volume (V_1). Added two drops of methyl orange indicator the colour turns yellow. Titrated against acid until the colour turns to orange yellow. Noted down the total volume (V_2). Alkalinity of water sample calculated as follows:

$$\text{Alkalinity of water sample} = \frac{v\times1000}{V}$$

Where V = Volume of water used
v = Volume of sodium thiosulphate used

6. Determination of Hardness of water

Diluted 25 ml of water sample to about with distilled water in flask. Added 1 ml of buffer solution. Added two drops of indicator solution, the solution turns wine red in colour. Added the standard EDTA titrant slowly with continuous stirring until the last reddish tinge disappears from the solution. The colour of the solution at the end point is blue under normal conditions. Noted down the volume of EDTA added. Calculated hardness of water as follows:

$$\text{Hardness of water sample as } CaCO_3 = \frac{1000\times v}{V}$$

Where v = Volume of EDTA used
V = Volume of water used

7. Determination of chloride in water

Took 50 ml water sample in a conical flask and added 2 ml of potassium dichromate solution. Poured 0.025N silver nitrate solution into the burette. Titrated the water sample with silver nitrate solution until reddish tinge appeared. Noted the end point (silver nitrate reacts with chloride ions and forms very slightly white ppt. of silver chloride). Calculation of chloride in water is done as follows:

$$\text{Chloride in water sample} = \frac{vol.of\ silver\ nitrate \times N \times 1000 \times 35.5}{V}$$

Where N = Normality of solvent
V = Volume of water sample

8. Determination of pH of water

Dipped the pH paper in the water sample. Compared the colour with that of the colour given on the wrapper of the pH paper book.

9. Determination of Fixed residue of water (Aneja, 2003)

An evaporating disc or beaker was taken, dried and weight till constant weight appeared.Water sample was filtered through whattman filter paper. Cleared filtrate was evaporated in the beaker in boiling water bath. Beaker was kept in oven for one hour at 105°C and final weight was taken.

B. Bacteriological examination (Aneja, 2003)

Bacteriological examination of water was done by Multiple Tube Fermentation Test in three tests:
1. Presumptive coliform test.
2. Confirmed coliform test.
3. Completed coliform test.

1. Presumptive Coliform Test

Labeled 5 double strength lactose broth tubes "10" and 5 single strength broth tubes "1" another 5 tubes "0.1". Added 10 ml water sample to the "10" labeled tube, 1 ml to "1" labeled tube and 0.1 ml to the "0.1" labeled tube aseptically. Incubated all 15 test tubes aerobically at 35°C for 48 hours.

2. Confirmed Coliform Test

Inoculated brilliant green lactose bile broth tubes with inoculums from all lactose broth positive presumptive tubes. Incubated all the inoculated tubes at 35°C for hours.

3. Completed Coliform Test

Streaked the two EMB agar plates from positive tubes with a sterile inoculating needle. Incubated the inoculated plates for 24 hour at 35°C in an inverted position.

C. Biochemical Tests (Aneja, 2003)

1. Indole Test

This test determines the ability of an organism to split indole from amino acid tryptophan. Ingredient were dissolved in distilled water and sterilized by autoclaving 121°C. 4-5 drops of Kovac´s reagent were slowly added to 24-48 hours. Growth and tube was shaken gently.

2. Methyl Red Test

This test is done to test the ability of the organism to produce and maintain stable acid conditions during the fermentation of glucose by overcoming action of the buffering solution and the pH of the organism is sustained about 4.5 and is detected by the change in the colour of methyl red indicator which is added at the end of the period incubation. The incubation was given to the culture for 24-48 hours.

3. Citrate Utilization Test

To determine if an organism is capable of utilizing as the sole source of carbon for metabolism result in alkalinity. With a straight inoculating loop colony was inoculated on Simmon´s medium slant.

4. Glucose Fermentation Test

To determine the ability of microorganism to degrade and ferment glucose with production of an acid and gas .Glucose broth was incubated with test microorganism and incubated overnight at 37°C.

RESULTS AND DISCUSSION

Total dissolved solids indicated the salinity behavior of groundwater. As per WHO, water containing more than 500 mg/lt of TDS is not considered desirable for drinking water. The experimental value for TDS of water sample was found to be 400 mg/lt which was less than WHO standard (Fig. 1), so this water was potable. Dissolved oxygen is important parameter in water quality assessment and reflects the physical and biological progress prevailing in the water.

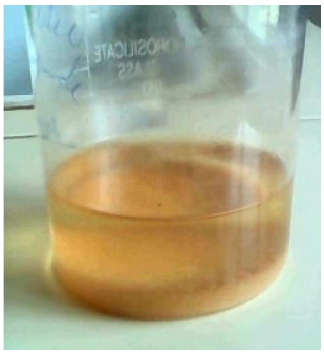
Table 1. Biochemical Tests of Bacterial Isolate

S.No.	Biochemical test	Results
1	Indole test	Negative
2	Methyl-Red test	Negative
3	Citrate Utilization test	Positive
4	Glucose fermentation test	Positive
5	Urease test	Positive

Table 2. Comparative estimation of experimental values with WHO standards

S. No.	Physico-chemical Properties	Experimental Values	W.H.O standard	Inference
1.	Total Dissolved solids	400 mg/lt	500 mg/lt	Potable
2.	Dissolved oxygen	2.52 mg/lt	7 mg/lt	Potable
3.	Biochemical oxygen demand	4.8 mg/lt	30 mg/lt	Potable
4.	Chemical oxygen demand	0.048 mg/lt	250 mg/lt	Potable
5.	Alkalinity	196 mg/lt	200 mg/lt	Potable
6.	Hardness	165.2 mg/lt	300 mg/lt	Potable
7.	Chloride	197.38 mg/lt	250 mg/lt	Potable
8.	pH	7	6.5 – 8.5	Potable
9.	Fixed residue	200 mg/lt	-	Potable

Fig. 1. Total Dissolved Solid in Water



Brown Precipitates

Fig. 2. Dissolved oxygen of Water Sample

Fig. 3. Biochemical Oxygen Demand of Water Sample

Fig. 4. Chemical Oxygen Demand of Water Sample

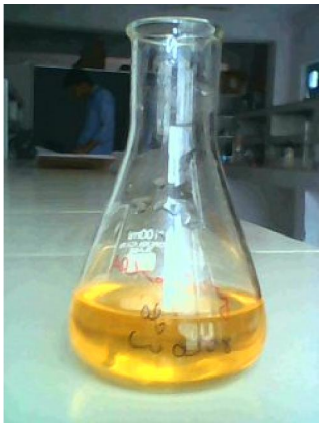


Fig. 5. Alkalinity of Water Sample

Fig. 6. Hardness of Water sample

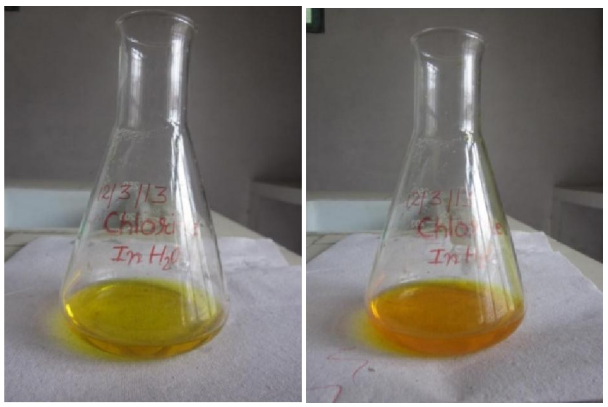


Fig. 7. Chloride in Water Sample

Fig. 8. Fixed residue of water sample

Bacteriological examination of water by multiple tube fermentation test

Fig. 9. Presumptive Coliform Test (Acid and gas production)

Fig. 9. Confirmed Coliform Test (Gas production)

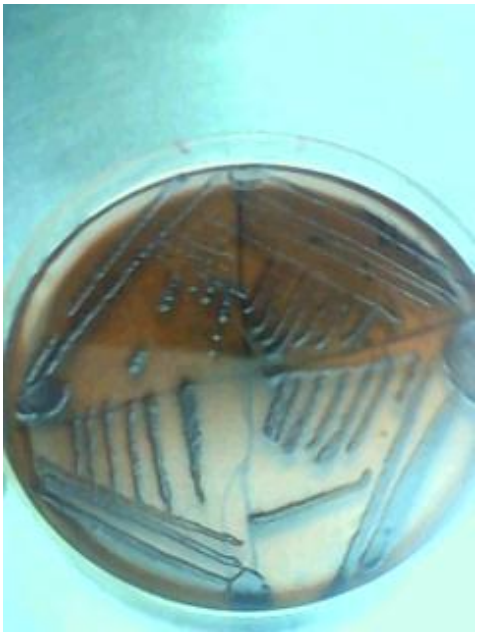


Fig. 10. Completed Coliform Test (Coliform colonies on EMB agar plate)

The DO values indicate the degree of pollution in water bodies. The experimental value for DO was 2.52 mg/lt which was less than WHO standard that is 7 mg/lt (Fig. 2). This indicated the potability of water. BOD is the amount of oxygen required by the aerobic bacteria to biochemically oxidizes the organic matter present in the waste. The high values of BOD suggest that oxygen present in water is consumed by aerobic bacteria´s which is present in water. Biological oxygen demand increases due to biodegradation of organic materials which exerts oxygen tension in a water body (Abida, 2008). The value for BOD was found to be 4.8 mg/lt. According to WHO the value of BOD should not exceed 30 mg/lt (Fig. 3). This showed that the water was potable. The alkalinity of water is due to the salt of carbonates, bicarbonates, borates, silicates and phosphates along with hydroxyl ions in the free state. Since most of the soaps have water softening agents such as washing soda and sodium carbonates, thus increases the concentration of carbonate hence alkalinity. COD is the amount of Chemical oxidant for the oxidation of organic matter present in the waste. High value of alkalinity indicates that the compound responsible for increase in alkalinity may work as chemical oxidant for COD and hence increase the value of COD. As per WHO, the values for alkalinity and COD were 200 mg/lt and 250 mg/lt, respectively, the experimental values for alkalinity and COD were found to be 196 mg/lt and 0.048 mg/lt (Fig. 4 and Fig. 5), which seemed to be lesser than WHO standard, hence the water was potable.

The hardness of water depends mainly on the presence of dissolved calcium and magnesium salts (Ikomi and Emuh, 2000). Hardness is mainly due to calcium or magnesium carbonate, bicarbonate, sulphates and chlorides. The experimental value for hardness was found to be 165.2 mg/lt (Fig. 6) which was more than WHO standard value that is 300 mg/lt, so water was safe for drinking. WHO standard value for chloride was 250 mg/lt. The higher value of chloride in water leads to various health problems. The chloride concentration serves as an indicator of pollution by sewage. The experimental value for chloride was found to be 197.38 mg/lt (Fig. 7). The

value was less than WHO standard value so water was potable. pH is a measure of the acidity or basicity of a solution. It is related with the molar concentration of the dissolved hydroxonium ions: a low pH (pH<7) indicates a high concentration of hydroxonium ions: while a high pH (pH>7) indicates a low concentration. This is a very important parameter in water quality assessment as it influences many biological and chemical processes within a water body. pH value was found to be 7 which lie in between WHO standard value (6.5 – 8.5) which showed the potability of water. Fixed residue denotes mainly the various kinds of minerals present in water sample. They do not contain any gaseous or colloidal fraction. They can be measured as residue left over after evaporation of filter sample. The experimental value for fixed residue was found to be 200 mg/lt (Fig. 8). Presumptive, confirmed and completed coliform tests were found positive as shown in Fig. 9 and 10. Biochemical tests are shown in Table 1 which depict that bacteria isolate showed negative for indole and methyl red test. Positive for citrate test, glucose test and urease test. These results showed that there were coliform (*Escherichia coli)* in water sample. All parameters were compared with WHO standard as per Table 2 as per URL: (http://www.who.int/water_sanitation_health/dwq/gdwq0506.pdf). Overall it was found that according to all physico-chemical parameters compared with WHO, water is potable. According to biological parameters, coliforms are present in water. Since local residents are using water from Majhwar stepwell for long time and no water borne diseases were reported. So by taking into account physico-chemical parameters and bacteriological examination, water was potable. More studies required to find strain of microbes present in stepwell water to check pathogenicity.

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