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Research Article

Memes-Interdisciplinary science journal, 2017 Volume 1 (1): 39 – 47

Physico-Chemical and Bacteriological Analysis of drinking well water samples around D.G.M.M.E.S Mampad College

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Abstract: In this study drinking well water samples were collected from four places around D.G.M.M.E.S Mampad College and have been analyzed for some physico-chemical parameters like pH, total hardness (TH), chloride (Cl^-) and fluoride (F^-) and bacteriological parameter like Coliforms (*E.coli*) analysis. The result revealed that pH, Cl^-, F^-, TH, and Coliforms ranged from 6.8 to 6.9, 20 to 50 mgL^{-1}, 0 mgL^{-1}, 8 to 14 ppm and 43 to 240 MPN respectively. All the parameters determined were within the standard drinking water quality values of WHO except Coliforms in all the samples. In general, the present investigation revealed that the maximum parameters were not at a level of pollution, but the presence of Coliforms prones that they are non-potable water without boiling.

Key Words: Physico-Chemical parameter; Bacteriological parameter; water quality; water quality standards

Acronyms Used

Cl-1: Chloride reagent 1 (Mercuric nitrate)

Cl-2: Chloride reagent 2 (Diphenyl carbazone indicator)

F-1: Fluoride reagent 1(TISAB: total ionic strength adjustment buffer 1)

TH-1: Total hardness reagent 1 (Calmagite indicator)

TH-2: Total hardness reagent 2 (Buffer solution of pH 2)

TH-4: Total hardness reagent 4 (Ethylnediaminetetraaceticacid (EDTA) solution)

BTB: Bromothimol blue

DSLB: Double strength lactose broth

SSLB: Single strength lactose broth

BGLB: Brilliant green lactose broth

EMB: Eosine methylene blue

CVI complex: Crystal violet-Gram's iodine complex

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Introduction

Life began in water and sustained by water. Water is one of the most important sources which human exploited more than any other resources for the existence of his life. Because of which many countries suffer from lack of access to safe drinking water [1]. The World Health Organisation [2] revealed that seventy five percent of all diseases in developing countries arise from consumption of polluted water. Therefore the quality of drinking water is most important. Quality of drinking water generally expressed in terms of its physical, chemical and bacteriological parameters [3]. Not only International agencies but also local agencies have established parameters to determine physico-chemical and bacteriological quality of drinking water [4]. Prolonged period of exposure to polluted drinking water cause major health problems [5]. As a universal solvent, water always carries other substances in it. Mahmoud et al. [6] also stated that the most common problems in household water supplies can be attributed to hardness, iron, sulfides, sodium chloride, alkalinity, acidity, and disease-producing pathogens, such as bacteria and viruses. Therefore, it is necessary to control the pollution in drinking water because the intake from other sources such as food or air may be difficult to avoid [7]. Like other areas in Malappuram district drinking water quality is major issue in places around D.G.M.M.E.S Mampad College. Therefore, the aim of this study was to examine the concentration of some physico-chemical and bacteriological parameters of drinking water around D.G.M.M.E.S Mampad college area.

Materials and Methods

Study Area

Drinking well water samples were collected from D.G.M.M.E.S Mampad College area which is the academic centre of the Mampad town, located at 8 km east Nilambur city of Malappuram district.

Sample Collection

Drinking well water samples were collected from four sampling sites (D.G.M.M.E.S Mampad College (MC), Pongalloor, Pulikkalodi and Rajeev Gandhi (RG) colony) of MC area. The potable water samples were collected in cleaned one litre polyethylene plastic bottles and brought to the laboratory in a well packed icebox to avoid unwanted change in quality of water. Before to the sampling all the bottles are washed and dried. Standard methods [8] were followed for sample collection and preservation.

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Instruments and Chemicals

The pH of all the samples determined using Systronics digital pH meter. And all the parameter except Coliforms determined on Octa Aqua kit for water analysis. All the chemicals used were commercially available from Merck and NICE.

Determination of Physico-Chemical and Bacteriological Parameters of Drinking Well Water

All the parameters except pH and Coliforms were determined by using water analyzing kit.

1. **Determination of pH**: The pH of each sample was measured using pH meter.
2. **Determination of Chloride**: 25 mL of each water sample was taken in a test bottle. And 5 drops of mercuric nitrate (Cl-1) reagent is added and mixed well until a distinct yellow colour develops. After that diphenyl carbazone indicator (Cl-2) is added drop wise, with constant shaking until the colour changes from yellow to red (end point). The number of drops of Cl-2 required for the colour change is noted. Finally, the concentration of chloride present in the sample was calculated using the Equation 1.

   $$Chloride\ (mgL^{-1}) = \ Number\ of\ drops\ \times 10 \qquad (1)$$
3. **Determination of Fluoride**: 5 mL of each water sample was taken in a test tube. And added 5 drops of Total Ionic Strength Adjustment Buffer-TISAB (F-1) then mixed well. The colour change that formed is compared with the fluoride colour chart and recorded the fluoride value.
4. **Determination of Total Hardness of Water (TH)**: 25mL of each water sample was taken in a test bottle to each of which 10 drops of TH-2 was added and mixed well. Then a few specs of TH-1 is added and mixed until a distinct pink colour developed. Finally drops of TH-4 were added, with constant shaking, until the colour changes from pink to blue. The number of drops of TH-4 required for the colour change is counted. Finally using Equation 2, total hardness of water sample was determined in ppm in terms of $CaCO_3$.

   $$Total\ hardness\ (ppm\ in\ terms\ of\ CaCO_3) = \ Number\ of\ drops\ \times 2 \qquad (2)$$
5. **Determination of Coliform Bacteria (*E.coli*)**: Coliform group of bacteria are characterized as gram negative, non-sporing, facultative bacteria that ferment lactose

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with the production of acid and gas within 48 hours of incubation at 37°C. Bacteriological analysis in water performed in three stages. They are, presumptive, confirmed and completed tests.

Before performing the first test, DSLB and SSLB were prepared and BTB was added as the indicator. And 10 mL of DSLB and SSLB solutions were taken in separate test tubes.

i. **Presumptive test**: 10mL of water sample was added to three DSLB tubes. Then added 1 mL of water sample to the first three set of SSLB and 0.1 mL of water sample to the last three set of SSLB. Also controls for each set of tubes were also included. Then Durham's tubes were inserted into each test tube in an inverted fashion. And all the tubes were incubated at 37°C for 24 hours. After 24 hours the tubes were examined and the numbers of tubes showing acid formation with at least 10% gas production were counted.
ii. **Confirmed test**: In this test a loopful of organisms from the positive lactose broth tube was incubated and streaked over EMB agar plates and Mac conkey agar plates and incubated at 37°C for 24 hours.
iii. **Completed test**: Here, the most typical colonies were selected from EMB agar plates and inoculated into lactose broth with Durham's tube and incubated at 37°C for 24-48 hours. Also sample from typical colonies in the EMB agar plates was taken and streaked over nutrient agar slant incubated for 24 hours at 37°C. After incubation periods the lactose broth were observed for gas production and gram staining was performed with colonies developed on agar slants.

Gram's staining: The bacterial smear was prepared on a velar glass slide. Dried in air and heat fixed. The smear was flooded with crystal violet solution for one minute. And the excess solution was poured off and washed with water. The smear was flooded with Gram's iodine for one minute and rinsed with water. Then the smear was decolourised with 95% ethanol in drop wise until no more violet colour comes out. And rinsed the smear with tap water. After counter staining the smear with Safranine for 60 seconds the smear was flooded with tap water. Dried the glass slide, and then observed under oil immersion objective.

Finally, their presence is expressed in terms of Most Probable Number (MPN). The MPN determination from Multiple Tube Test is given in Table 1.

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Table 1: MPN determination from Multiple Tube Test

Number of Tubes Giving Positive Reaction out of			MPN index per 100 mL	95% Confidence limits	
3 of 10 mL each	3 of 1 mL each	3 of 0.1 mL each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	336	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

From: Standard Methods for the Examination of Water and Wastewate, 14th edition. American Public Health Association, American Water Work Association, Water Pollution Control Federation, Washington, D.C., 1975.

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Results and Discussion

Values of the measured physico-chemical and Bacteriological parameters of the drinking well water samples are shown in **Table 2**.

Table 2: Values of Physico-Chemical and Bacteriological parameters of well water samples under study.

	MC	Pongalloor	RG Colony	Pulikkalodi
pH	6.8	6.9	6.88	6.9
Chloride (mgL^{-1})	50	20	30	30
Fluoride (mgL^{-1})	0	0	0	0
Total Hardness (ppm)	10	8	10	14
Coliforms (MPN)	240	43	240	43

1. **pH**

The pH of water is an indication of the hydrogen ion concentration in water. Drinking water with a pH between 6.5 to 8.5 is generally satisfactory. If the pH is below 6, water will be acidic in nature and tend to be corrosive. pH above 8.5 water will be alkaline in nature and less corrosive but tend to have an unpleasant taste. In this study, the concentration of hydrogen ion (pH) ranges between 6.8 to 6.9 and all the water samples analyzed have a concentration within the desirable limit standard set by the WHO. Thus indicated that the measured pH values of the drinking water samples were within the permissible value of WHO; which will not impart any harmful effect to the consumers.

2. **Chloride**

The higher concentration of chloride in drinking water is the indication of sewage pollution and also imparts laxative effect. Atmospheric sources or sea water contamination is a reason for the bulk of the chloride concentration in groundwater which may exceed due to base-exchange phenomena, high temperature, domestic effluents, septic tanks and low rainfall [9]. Porosity and permeability of soil also a reason for building up the chlorides concentration [10]. The chloride concentration of studied water samples were within permissible limit of 250 mgL^{-1} prescribed by

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WHO [11]. In the present study, the results of chlorides in all sampling sites ranged from 20 to 50mgL^{-1}.

3. **Fluoride**

Fluorides are derived from fluorine. It is found in many rocks and minerals in the soil and enters the water as the water passes through these soils. In this study, the measured value of F^- ion of the water samples were 0.00 mgL^{-1}. So all the samples were within the maximum standard value of F^- in drinking water set by WHO (1.5 mgL^{-1}).

4. **Total hardness (TH)**

Hardness is the property of water which prevents lather formation with soap. In groundwater, the sources of hardness are bicarbonates, carbonates, sulphates and chlorides of calcium and magnesium. So, the principal hardness causing ions are calcium and magnesium. The acceptable limit of total hardness is 300 mgL^{-1} whereas the maximum limit is 600 mgL^{-1}. The hardness of analyzed water samples varied from 8 to 14 ppm as $CaCO_3$. The highest value of total hardness was observed at Pulikkalodi sampling site, as shown in **Table 2**. And all the TH values were within the acceptable limit values of BIS (300 ppm) and WHO (500 ppm) and hence not hard in nature.

5. **Coliforms (*E.coli*)**

E.coli is considered as an indicator organism for determining bacteriological quality. Its presence and numbers indicate the possibility and extent of contamination respectively. In this study, the presence of Coliforms like E.coli is observed in all the well water samples, ranging from 43 to 240 MPN. The higher number of Coliforms in RG colony (240 MPN) and Pulikkalodi (240 MPN) sampling site may be attributed to the unhygienic sanitation and unclean surroundings around the wells.

Conclusion

In this study, the collected drinking well water samples around D.G.M.E.S Mampad College were analyzed for physico-chemical and bacteriological parameters of pH, Cl^-, F^-, TH, and Coliforms. The result showed that all the measured parameters were within the standard drinking water quality given by WHO except Coliforms concentration. In general, the present investigation found that the maximum parameters were within the desirable and

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permissible limit hence may not cause harmful effect to the consumers. But the presence of Coliforms prones that they are not potable without boiling.

Acknowledgement

Thanks for Food Technology department for providing Microbiology lab facilities and support.

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