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Bacteriological Analysis Of Drinking Water Collected In Autumn Season From Vellore District, Tamil Nadu, India

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Abstract

The present study was aimed to assess the potability of Drinking water during the Autumn season (October 2018 to November 2018) in five different locations of Vellore district, Tamil Nadu, India. The physico-chemical characteristics of Drinking water samples which are collected from different locations of Vellore district were analyzed and it was observed that except the fluoride content all the other parameters are under permissible limit of TNPCB. So, finally we found that, physico-chemically the water does not contain more hazardous compounds and safe for drinking purposes. The bacterial population in the drinking water was enumerated. The population of bacteria is within the limit so biologically the collected are not injurious to human health. The drinking water collected in different locations of Vellore district was assessed by the water potability test, Most Probable Number (MPN). It was clearly showed that the bacterial population was very low at 24 hours incubation so not showing any colour change but after 48 hours incubation, the bacterial population was increased in the Brilliant Green Lactose Broth (BGLB) and colour change was observed. In conclusion, the water sample contains the normal bacterial population but not the coliform bacteria *Escherichia coli*. So, the drinking water collected from five different locations of Vellore district are potable to drink during Autumn season.

Keywords: Autumn season, Drinking water, Physico-chemical characteristics, Water potability and MPN Technique.

1 Introduction

Water is considered as a universal solvent and it plays an important role in the life of all living organism. Next to Oxygen, every living things need water to continue their life and the fresh water which is used for drinking purpose is mostly from ground water. It was estimated that about 98 % of the drinking water which was used by the peoples nowadays was from ground water [1]. In human body, water helps in releasing out of toxic and waste products and it carries out various function such as aid in digestion, maintaining the body heat balance, regulating the metabolism and for the proper functioning of the cells. Approximately about 60 % of the human system is made of water whereas infants and children have greater percentage of water in their body when compared to adults. Water has many trace elements which cannot be synthesised by our own human system. These elements are very useful for the growth and biological activity of the body. Any alteration in the trace elements of the drinking water may lead to change in the nature of water and results in diseased condition for the living organism [2].

In Indian states like Tamil Nadu, the peoples are using the Groundwater for their household domestic purposes, agricultural irrigation and industrial usage. Peoples who are living in the rural areas of India are completely depending on the ground water for their domestic usage and irrigation of agricultural crops. The major sources of water are seasonal rainfall, rivers, lakes, streams, wells and bore wells.

Mostly in present days, drinking water is frequently getting polluted in multiple ways because of human activities such as industrialisation, urbanization, improper drainage system, disposing the industrial wastes directly into the water bodies without proper treatment and also due to the population growth and their activities. All the above said human activities make the water bodies highly contaminated and makes unfit for drinking purpose. In most of the industrial areas, the water gets polluted mainly because of the drainage which comes out from the industries such as chemical, leather, textile, paper mill, nuclear power plant and other industries. These factors make big issue of water pollution making it no longer used for drinking purpose [3].

Earth surface is covered about 70 % of water and from that huge percentage, the percentage of water used for human consumption is about 0.002 % only. Many infectious and dangerous microbial diseases are spread through contaminated drinking water. Infections

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transmitted through the water are called as Water borne diseases and the selected Bacteria, Virus and Protozoa are considered as the Water borne microorganisms. Some of the most familiar Water borne diseases includes Amoebiasis (*Entamoeba histolytica*), Malaria (*Plasmodium vivax*), Cholera (*Vibrio cholerae*), Dysentery (*Shigella dysentriae*), Paratyphoid fever (*Salmonella paratyphi*), Typhoid (*Salmonella typhi*) and Jaundice (Hepatitis B virus) are caused to humans. Harmful carcinogenic chemicals such as Arsenic, Fluorides, Lead and Nitrates which are present in the poor sanitary water and it have a negative effect on the health of humans, animals, plants and aquatic living organisms [4].

Industrial wastes are discharged into water bodies without any treatment contains large amount of organic matter and also contaminants as petroleum hydrocarbons, acids, alkalis and dyes [5].

The menace of microbial contamination of drinking water is associated with faecal contamination as a result of excretion of sewage waste into water resources. Most of the Protozoan diseases (Amoebiasis, Giardiasis, Balantidiasis, etc.) and Helminthic diseases (Trichuriasis, Teniaisis, Ascariasis, etc.) are associated with the faecal contamination of drinking water.

On the authority of WHO in 2008, the mortality rate federated with water borne diseases was more than 5 million people per year. Recent report of World Health Organization has showed that every year about 1.6 million children are died because of water borne diseases. The seasonal variations are having an important connection with the microbial contamination of drinking water [6].

The quality of the drinking water has been determined by checking the presence of Coliforms. The coliform is a type of bacteria that can act as a microbial quality indicator of drinking water. Most of the coliforms are belonged to the family Enterobacteriaceae, the most common intestinal pathogenic bacteria.

The well known example for coliform is the Gram negative, rod shaped, Catalase and Oxidase producing motile bacteria *Escherichia coli*. Other coliform bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae* and *Citrobacter* sp. are also present in the water but the presence of that coliforms are not sincerely considered when compared to *Escherichia coli* presence [7].

It was estimated that the House hold water treatment can reduce Diarrhoea by 30 – 40 % [8]. Most frequently used house hold water treatment is using the boiled water for drinking purpose but very effective results is shown in using ceramic and biosand household water filters.

They have the highest potential in improving the quality of water and in decreasing the water borne diseases and death. For large scale usage, drinking water is treated by disinfection method with the use of disinfectants like Chlorine along with Sodium or Calcium hypochlorite to make it fit for drinking [9].

2 Experimental

2.1. Locations selected for the sampling of Drinking water

Five different locations in Vellore district was selected for the collection of Drinking water samples. The selected locations are: Ambur, Jolarpet, Tirupattur, Vaniyambadi and Vengalaburam.

2.2. Collection of Drinking water samples

Drinking water samples was collected in clean sterilized bottles from water source in Vellore district of Tamil Nadu after the tap was allowed to run for 5 minutes. After sampling of Drinking water samples, the collected samples were transported to laboratory for physico-chemical and microbiological testing. The sampling of Drinking water was done in Autumn season (October 2018 to November 2018).

2.3. Analysis of Physical characteristics of Drinking water

2.3.1. Colour

The colour of the collected Drinking water samples was observed visually.

2.3.2. Odour

The odour of the collected Drinking water samples was categorized as pleasant or unpleasant by direct smelling of the sample.

2.3.3. Temperature

The temperature of the Drinking water samples were noted using Thermometric method at the site of sampling using portable calibrated mercury thermometer.

2.3.4. pH

The pH of the Drinking water samples was determined by Potentiometric method using pH meter already standardized by using buffer solutions of known value before analysis.

2.3.5. Electrical conductivity (EC)

Electrical conductivity of the Drinking water samples was determined by conductivity meter following the procedure of Richard [10].

2.3.6. Total Suspended solids (TSS)

Total suspended solids are (TSS) of the Drinking water samples was determined by using following formula of Anon [11].

$$TSS = \frac{(Final\ weight - Initial\ weight)}{Ammount\ of\ sample\ taken} \times 1000$$

2.3.7. Total Dissolved Solids (TDS)

Total dissolved solids (TDS) of the Drinking water samples were determined following the procedure of Richard [10] by using Electrical Conductivity (EC) meter.

$$TDS = \frac{Electrical\ Conductivity}{0.67}$$

2.3.8. Total Hardness

For the analysis of Total hardness in Drinking water samples, 25 ml of sample was diluted to 50 ml with distilled water. A volume of 1 to 2 ml of buffer was added to give a pH of 10.0 to 10.1. One to two drops of indicator solution was added and titrate with EDTA titrant to change

in colour from reddish tinge to blue. A sample volume that requires less than 15 ml EDTA titrant was selected and complete titration will be done within 5 min after buffer addition. The EDTA titrant was standardized against standard calcium solution using the above procedure.

Total Hardness (mg $CaCO_3$/L) = A × B × 1000/ ml sample

Where, A = ml EDTA titrated for sample; B = mg $CaCO_3$ equivalent to 1 ml EDTA titrant

2.3.9. Estimation of Biological Oxygen Demand (BOD)

The Biological Oxygen Demand (BOD) of the Drinking water samples was estimated by Winklers Iodometric method [12].

2.3.10. Estimation of Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) of the Drinking water samples was estimated by Titrimetric method [12].

2.4. Analysis of Chemical characteristics of Drinking water

2.4.1. Estimation of Calcium and Magnesium

The Calcium and Magnesium content of the Drinking water samples was estimated by EDTA Titrimetric method [12].

2.4.2. Estimation of Chloride

The Chloride content of the Drinking water samples was estimated by Silver nitrate Titrimetric method [13].

2.4.3. Estimation of Sodium and Potassium

The Sodium and Potassium content of the Drinking water samples was estimated by Flame photometric method [13].

2.4.4. Estimation of Sulphate

The Sulphate content of the Drinking water samples was estimated by Turbidimetric method [12].

2.4.5. Estimation of Nitrogen

The Nitrogen content of the Drinking water samples was estimated by Titrimetric method [12].

2.4.6. Estimation of Phosphorus

The Phosphorous content of the Drinking water samples was estimated by Spectrophotometry method [13].

2.4.7. Estimation of Zinc, Iron, Copper, Lead, Chromium and Manganese

The presence of Zinc, Iron, Copper, Lead, Chromium and Manganese in the Drinking water samples were estimated by Atomic Absorption Spectrophotometric (AAS) method.

2.5. Enumeration of Bacterial population in Drinking water

The bacterial population in the collected Drinking water samples will be enumerated in the Standard Plate Count Agar plates by Standard Plate Count (SPC) method. The Drinking water sample collected from five different locations of Vellore district was serially diluted upto 10^{-6} dilution to determine the bacterial population.

A volume of 0.1 ml from the sample dilutions (10^{-4} and 10^{-5}) were spreaded (Spread plate technique) on sterile petriplates containing Standard Plate Count Agar for the growth of bacterial colonies at 37 ˚C for 24 hrs.

The numbers of bacterial colonies in the Standard Plate Count Agar plates were counted and calculated by using the formula:

$$cfu/ml = \frac{No.of\ colonies\ counted}{Amt.of\ sample\ taken} \times Dilution\ Factor$$

2.6. Determination of Potability of Drinking water by Most Probable Number (MPN) Technique

The Most Probable Number (MPN) Technique was used to check the potability of the collected Drinking water. The MPN technique contains three steps *viz*., a) Presumptive test, b) Confirmed test and c) Completed test.

a) Presumptive test

Presumptive test involves the primary presumption for the presence of Gram negative coliform bacteria in the samples demonstrated by the appearance of gas in the Brilliant Green Lactose Broth (BGLB). For the presumptive test procedure, 15 sets of test tubes containing BGLB required for each sample under analysis. Each test tube contained 10 ml of BGLB and inoculated with the water sample in a sequential order of 10 ml in three of each Double Strength BGLB, 1 ml in three of each Single Strength BGLB and lastly 0.1 ml in three of each 10 ml Single Strength BGLB. All the test tubes were incorporated with Durham's tubes for detection of gas formation by Gram negative coliform bacteria. Test tubes were incubated in an Incubator at 37 °C for 24 hours and 48 hours.

b) Confirmed test

Positive samples with the production of gas in the BGLB were selected for the confirmed test procedures to detect the indicator bacteria of fecal origin *Escherichia coli.* The Eosin Methylene Blue (EMB) agar media was used to differentiate other Gram negative coliform bacteria from the *Escherichia coli* by the production of Green metallic sheen in the EMB medium. The presence of Green metallic sheen in EMB confirms the presence the indicator bacteria *Escherichia coli*. One loopful sample from the positive test tubes was inoculated on EMB by streaking and incubated at 37 °C for 24 hours and then observed for the production of Green metallic sheen.

c) Completed test

From the positive EMB plates showing Green metallic sheen colonies of *Escherichia coli*, the isolated colonies were observed microscopically for their Gram reactions. This was the final stage of the MPN method where in the decision of water quality as potable or non-potable, could be made after confirmation and completion of the study. Finally, the standard biochemical tests were performed to confirm the identification of all the pathogenic isolates found in all the collected drinking water samples.

3 Results and Discussion

The present study was aimed to study the effect of seasonal variations on the presence of bacterial coliforms and potability of drinking water which are collected from five different locations of Vellore district *viz*., Tirupattur,

Vengalaburam, Jolarpet, Vaniyambadi and Ambur. The research was carried out in Autumn (October 2018 to November 2018). The findings of the present research are discussed here.

3.1. Physico - chemical characteristic of collected Drinking water samples in Autumn season

The drinking water samples which are collected from the different locations of Vellore district and its physico-chemical properties was analysed during the Autumn season (October 2018 - November 2018) and the findings were tabulated in the Table 1. The results of the physico-chemical characteristic were compared with the Standard table of Tamil Nadu Pollution Control Board (TNPCB). The physical and chemical characteristic of the collected drinking water are within the permissible limit of TNPCB Standard except the excess content of Fluoride. In physical examination, the water samples are colourless, odourless, and alkaline in pH (6.9 - 7.5). The temperature of water samples was ranging from 18 °C - 20.7 °C. Other physical characteristic values are ranging from Electrical conductivity (242 - 266 dSm^{-1}), Total suspended solids (110 -155 mg/L), Total dissolved solids (53 – 75 mg/L), Hardness (189 - 242 mg $CaCO_3$/L), Biological oxygen demand (10.5 - 24.9 mg/L), Chemical oxygen demand (83 - 125 mg/L). The recorded value ranges for chemical parameters in collected water are, Calcium (20 - 54 mg/L), Magnesium (18 - 38 mg/L), Chloride (240 - 350 mg/L), Fluoride (1.7 - 2.4 mg/L), Sodium (233 - 300 mg/L), Potassium (64 - 103 mg/L), Sulphate (6.30 -9.80 mg/L), Nitrate (25.4 - 38.6 mg/L), Phosphorus (6.3 - 8.9 mg/L), Zinc (0.015 - 0.030 mg/L), Iron (0.05 - 0.09 mg/L), Copper (0.015 - 0.033 mg/L), Lead (0.0011 - 0.008), Magnesium (26.71 - 39.30 mg/L) and Chromium (0.003 - 0.010 mg/L). It was observed that except the fluoride content all the other parameters are under permissible limit of TNPCB. So, finally we found that, physico-chemically the water does not contain more hazardous compounds and safe for drinking purposes.

Table 1: Physico – chemical characteristics of collected drinking water samples in Autumn season (October 2018 – November 2018)

Physico - chemical properties	Drinking water sample collected Locations					Standard by TNPCB
	Tirupattur	Vengalaburam	Jolarpet	Vaniyambadi	Ambur	
Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Temperature (°C)	18.00	17.5	18.3	20.7	19.5	40
pH	6.9	7.2	7.4	7.6	7.5	5.5 – 9.0
EC (d Sm^{-1})	251	242	253	275	266	1500
TSS (mg/L)	126	110	133	155	147	200
TDS (mg/L)	62	53	66	83	75	200
Hardness (mg $CaCO_3$/L)	202	189	210	242	240	250
BOD (mg/L)	14.9	10.5	17.3	24.9	22.1	30
COD (mg/L)	92	83	103	125	111	250
Calcium (mg/L)	28	20	42	54	48	200
Magnesium (mg/L)	20	18	26	38	31	50
Chloride (mg/L)	240	225	260	350	298	600
Fluoride (mg/L)	2.1	1.7	1.9	2.4	2.2	1
Sodium (mg/L)	252	233	270	300	295	600
Potassium (mg/L)	79	64	88	103	98	250
Sulphate (mg/L)	7.20	6.30	7.76	9.80	8.50	12
Nitrate (mg/L)	28.7	25.4	32.4	38.6	26.5	600
Phosphorous (mg/L)	6.8	6.3	7.3	8.9	7.7	10
Zinc (mg/L)	0.017	0.015	0.020	0.030	0.025	0.01
Iron (mg/L)	0.06	0.05	0.07	0.09	0.08	0.2
Copper (mg/L)	0.028	0.033	0.022	0.015	0.017	0.01
Lead (mg/L)	0.008	0.006	0.0011	0.015	0.012	0.05
Magnesium (mg/L)	30.60	26.71	33.78	39.30	35.45	50
Chromium (mg/L)	0.006	0.003	0.007	0.010	0.008	0.01

[EC – Electrical conductivity; TSS – Total Suspended Solids; TDS – Total Dissolved Solids; BOD – Biological Oxygen Demand; COD – Chemical Oxygen Demand; TNPCB – Tamil Nadu Pollution Control Board; cfu – Colony forming unit]

Table 2: Enumeration of Bacterial population in collected drinking water samples during Autumn season (October 2018 – November 2018)

Drinking water sample collected Locations	Bacterial population	
	× 10^4 cfu/ml	× 10^5 cfu/ml
Tirupattur	70	62
Vengalaburam	65	60
Jolarpet	85	73
Vaniyambadi	90	81
Ambur	76	69

3.2. Enumeration of Bacterial population in collected Drinking water samples during Autumn season

The bacterial population in the drinking water collected from different locations of Vellore district in Autumn season (October 2018 - November 2018) was enumerated and results were furnished in the Table 2. The bacterial colonies in Standard plate count agar are counted in two dilutions (10^{-4} and 10^{-5}) by using Quebec colony counter and the results are expressed as colony forming unit/ml (cfu/ml). As like in the previous Rainy season, more bacterial population was recorded at 10^4 dilution than 10^5 dilution. Highest bacterial population was recorded in Vaniyambadi (90 × 10^4 cfu/ml) followed by Jolarpet (85 × 10^4 cfu/ml), Ambur (76 × 10^4 cfu/ml), Tirupattur (70 × 10^4 cfu/ml). Lowest bacterial population was seen in Vengalaburam (65 × 10^4 cfu/ml). The population of bacteria is within the limit so biologically the collected are not injurious to human health.

3.3. Presumptive test of MPN test in Autumn season (October 2018 – November 2018)

The drinking water collected in different locations of Vellore district was assessed by the water potability test, Most Probable Number (MPN) Technique and the results are furnished in Table 3. As like in the previous Rainy season, in this season also the results are studied in both 24 hours and 48 hours incubation. Like previously tested Rainy season, the Single strength Brilliant Green Lactose Broth (BGLB) tubes (10 ml BGLB + 0.1 ml water sample and 10 ml BGLB + 1 ml water sample) does not showed any positive reaction but the double strength Brilliant Green Lactose Broth (BGLB) tubes (10 ml BGLB + 10 ml water sample) has showed positive reactions in two locations be after 24 hours of incubation. Those two locations are Tirupattur and Jolarpet which showed positive reaction in both 24 hours and 48 hours incubation. The Tirupattur water sample has shown MPN Index – 8 /100 ml of water after 24 hours incubation and MPN Index –220 /100 ml of water after 48 hours incubation. The water sample collected from Jolarpet has shown MPN Index – 8/100 ml of water after 24 hours incubation and MPN Index – 210/100 ml of water after 48 hours incubation. The water samples collected from Ambur, Vengalaburam and Vaniyambadi does not shows positive reaction (no acid and gas production in BGLB) (MPN Index - 8/100 ml of water) in any MPN tubes in 24 hours. After 48 hours incubation, the water samples collected from Ambur (MPN Index -17/100 ml of water), Vengalaburam (MPN Index - 17/100 ml of water) and Vaniyambadi (MPN Index - 14/100 ml of water). It has showed the acid production in tubes with yellow colour formation. It clearly showed that the bacterial population was very low at 24 hours incubation so not showing any colour change but after 48 hours incubation, the bacterial population was increased in the BGLB and colour change was observed.

3.4. Confirmed test of MPN test in Autumn season (October 2018 – November 2018)

The findings of Confirmed test which is considered as the second step of Most Probable Number (MPN) technique in Autumn season (October 2018 to November 2018) was presented in Table – 4. A loopful of BGLB sample from the Presumptive test tubes was streaked on the Eosin Methylene Blue (EMB) and incubated in an Incubator at 37 °C for 24 hours and 48 hours. After incubation, the EMB plates showed the presence of bacterial colonies but the Green metallic sheen was not observed. Hence, we concluded that the water sample contains the normal bacterial population but not the coliform bacteria *Escherichia coli*. So, the drinking water collected from five different locations of Vellore district are potable to drink during Autumn season.

Table 3: Results of Presumptive test of MPN test in Autumn season (October 2018 – November 2018)

S. No	Location	Incubation period	Combination of Positives			* MPN Index/ 100 ml	** 95 % Confidence Limits	
			3 of 10 ml	3 of 1 ml	3 of 0.1 ml		Lower	Upper
1	Tirupattur	24 Hours	3	0	0	8	2	22
		48 Hours	3	3	2	220	70	440
2	Vengalaburam	24 Hours	3	0	0	8	2	22

		48 Hours	3	2	1	17	7	40
3	Jolarpet	24 Hours	3	0	0	8	2	22
		48 Hours	3	2	2	210	35	370
4	Vaniyambadi	24 Hours	3	0	0	8	2	22
		48 Hours	3	2	0	14	6	35
5	Ambur	24 Hours	3	0	0	8	2	22
		48 Hours	3	2	1	17	7	40

[*** MPN Index –** Referred Standard MPN Table, **** 95 % Confidence Limits –** Referred Standard MPN Table. All the experiments have been done 3 times and one representative data have been shown]

Table 4: Results of Confirmed test of MPN test in Autumn season (October 2018 – November 2018)

S. No	Location	Incubation period	Growth on EMB plate	Production of Metallic sheen on EMB plate	Potability of Drinking water
1	Tirupattur	24 Hours	+	-	Potable
		48 Hours	+	-	
2	Vengalaburam	24 Hours	+	-	Potable
		48 Hours	+	-	
3	Jolarpet	24 Hours	+	-	Potable
		48 Hours	+	-	
4	Vaniyambadi	24 Hours	+	-	Potable
		48 Hours	+	-	
5	Ambur	24 Hours	+	-	Potable
		48 Hours	+	-	

[**+ :** Present and **- :** Absent; All the experiments have been done 3 times and one representative data have been shown]

4 Conclusions

The present study concludes that the Drinking water collected in Autumn season (October 2018 to November 2018) from five different locations around Vellore district, Tamil Nadu, India (Ambur, Vaniyambadi, Tirupattur, Jolarpet and Vengalaburam) was potable to drink. The water samples do not contain any harmful bacterial isolates which are very injurious to human health and main causative agent for many Water borne transmitted diseases. But, the water collected from these five locations shows presence of little number of bacterial populations which are not harmful and it may be beneficial to the living organism water shows variations in Autumn season (October 2018 to November 2018). So, it is recommended to boil the water for better sanitation and to avoid spreading of Water borne diseases.

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