Assessment of Faecal Contamination in Regularly using Borehole Water of Kanyakumari District, Tamilnadu

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ABSTRACT

Groundwater is one of the important water sources in India accounting for 63% of all irrigation water and over 80% of rural and urban domestic water supplies. As ground water moves very slowly, usually contamination remains undetected for longer periods. In the present study, 5 ground water samples were collected and microbiological evaluation was done to understand the efficacy of water. Among the 5 samples analysed, only 1 water sample was contaminated with bacteria. Microscopical, macroscopical and biochemical tests were performed to detect of Escherichia coli. MPN test was performed to evaluate the most probable number of bacteria in the water. MPN index was found to be 4, which is not acceptable by World Health Organization. Hence, it was evaluated that the sample III is hydraulically connected to the septic-tank field lines and the leakage from pipelines of septic tank has been established to be a major source of groundwater pollution with maximal microbial communities. Therefore, it is advised that for borehole water to be potable it should be treated by chlorination or other treatment method suitable for public health. Also, permanent monitoring network should be developed at least once in a year to check the microbial communities.

Keywords: *Bacteria, Escherichia coli, MPN index, Water contamination,*

1. Introduction

Natural groundwater in most part of the world is uncontaminated. Recent studies implies that there is possibility of uncleanness of ground water through anthropogenic activities. These have resulted in changes in the physical and biochemical characteristics of ground water, making it difficult to meet the needs of the intended users. The typical sources of wastewater entering septic tanks include black water from toilets (approximately 38%), grey waters from laundry (25%), baths (22%) and kitchen sinks (15%). A standards septic tank is so designed to manage sewage inhabiting bacteria, viruses, nitrate, inorganic and other organic compounds [1,2, 3].

Drinking water is crucial for maintaining a healthy human life. Waterborne diseases are a leading cause of death in many regions and represent a significant economic burden to

everyone. The quality of water is often declining because of population growth, continuous industrial development, and also inadequate sanitation. [4].

The coliform bacteria are rod-shaped, anaerobic, gram-negative, non-spore-forming organisms of Enterobacteriaceae family. The World Health Organization (WHO) recommends that there must be zero coliforms in a 100 ml water sample [5]. Faecal coliforms are a subgroup of total coliforms, and their presence in water ensures the presence of faecal contamination. Waterborne diseases that are associated with faecal coliform contamination are mainly ear infections, dysentery, typhoid, bacterial and viral gastroenteritis, and hepatitis A [6, 7]. *Escherichia* coliforms always associated with faecal contamination of water. In human faeces, 95% of coliforms are *Escherichia coli* [8, 9]. The presence of *E. coli* leads to diarrhoea and other gastrointestinal illnesses. One severe condition caused by *E. coli* is haemorrhagic colitis, which is characterized by the abdominal cramps, watery diarrhoea, and lower intestinal bleeding, that may sometimes be accompanied by fever and vomiting [10].

2. Materials and Methods

2.1 Sample Collection

Five bore well water samples were collected from different village areas of Kanyakumari District Tamilnadu. Sampling was done between the hours of 5:00 am and 8:00 am. It must be clearly noted that the septic tanks should be within 0 to 35 m. A control borewell was selected, in which the radius of well and septic tank is compulsory above 35m. After collection, the samples were kept in sterilized bottles and transported immediately to the laboratory for further analysis.

2.2 Total Bacterial Count

Using aseptic technique, the initial dilution is made by transferring 1 ml of water sample to a 99 ml sterile saline blank (1/100 or 10^{-2} dilution). Immediately after the 10^{-2} dilution has been shaken, uncap it and aseptically transfer 1 ml to a second 99 ml saline blank. Since this is a 10^{-2} dilution, this second blank represents a 10^{-4} dilution of the original sample. Shake the 10^{-4} dilution vigorously and transfer 1 ml to the third 99 ml blank. This third dilution represents a 10^{-6} dilution of the original sample. Repeat the process once more to produce a 10^{-8} dilution. Shake the 10^{-4} dilution again and aseptically transfer 1.0 ml to one petri plate and 0.1 ml to another petri plate. Do the same for the 10^{-6} and the 10^{-8} dilutions. Remove one agar pour tube from the 48º to 50ºC water bath. Carefully remove the cover from the 10^{-4} petri plate and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a circular motion while it rests on the tabletop. Repeat this process for the remaining five plates. After the pour plates have cooled and the agar has hardened, they

are inverted and incubated at 25ºC for 48 hours or 37ºC for 24 hours. At the end of the incubation period, count the colonies on each plate with Quebec colony counter should be used. Calculate the number of bacteria (CFU) per ml of sample by dividing the number of colonies by the dilution factor multiplied by the amount of sample added to liquefied agar [11].

Number of colonies (CFUs) = number of bacteria/ ml

dilution X amount plated

2.3 Determination of Growth Curve

An over-night culture of *E. coli* is inoculated in 100 ml of nutrient broth in a 250 ml conical flask. The flask containing culture was incubated in an orbital shaker at 37°C, 180 rpm. Aliquots of the culture were taken aseptically at regular intervals and the turbidity was measured in a spectrophotometer at 600 nm using nutrient broth as blank. Optical density of the samples at 600 nm was recorded till 28 hrs of growth. The O.D 600 values as a function of time were plotted in a semi-log paper to generate the growth curve.

2.4 Identification of Bacteria

The bacteria were identified by microscopic, macroscopic and biochemical tests [12].

2.5 MPN Test

2.5.1 Presumptive Test

Prepare Lactose broth of single and double strength in test tubes with Durham's tube and autoclave it. Take three sets of test tubes containing five tubes in each set; one set with 10 ml of double strength (DS) other two containing 10 ml of single strength (SS). Using sterile pipettes, transfer 10 ml of water to each of the DS broth tubes. Transfer 1 ml of water sample to each of 5 tubes of one set of SS broth and transfer 0.1 ml water to five tubes of remaining last set of SS broth tubes. Incubate the tubes at 37°C for 24 hours. After incubation, observe the gas production in Durham's tube and the colour change of the media. Record the number of positive results from each set and compare with the standard chart to give presumptive coliform count per 100 ml water sample.

2.5.2 Confirmed Test

Some microorganisms other than coliforms also produce acid and gas from lactose fermentation. In order to confirm the presence of coliform, a confirmatory test is done. For this, a loopful of suspension from a positive tube is inoculated into a 3 ml lactose-broth or brilliant green lactose fermentation tube and to an agar plate (EMB agar) or slant.

A. Inoculation of the lactose-broth

Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen, further incubate up to a maximum of 48 ±3 hours to check gas production.

B. Inoculation in media slants

Take a loopful of suspension from a positive tube and inoculate it on the agar surface. The agar slants should be incubated at 37°C for 24± 2 hours. Colonies must be examined macroscopically.

2.5.3 Completed Test

Transform a typical coliform colony from the agar plate into a tube of brilliant green bile broth with placed Durham's tube and on the surface of a nutrient agar slant. Incubate at 35°C for 24 hours. After 24 hours, check the broth for the production of gas, and perform Gram staining for organisms on the nutrient agar slant.

3. Results and Discussion

Water samples were collected from the boreholes of different villages of Kanyakumari District and are mentioned in Table I. The depth of borehole and the surface distance of borehole to septic tank of sampling site villages were also mentioned in Table 1. The minimum depth of borehole should be 50 m and the surface distance of borehole to septic tank must be 35m. But the sampling site III does not coexist with the minimal range, as the depth is 40m and surface distance is 25 m.

Table I: Sample Collection Site

Sample Number	Collection Site	Depth of Bore Hole (m)	Surface Distance: Borehole to Septic Tank (m)
I	Anjugiramam	100	60
II	Puthalam	140	65
III	Manavalakurichi	40	25
IV	Kaliyakavilai	105	70
V	Vanniakudi	120	65

3.1 Total Bacterial Count

Total bacterial count (TBC) was done manually in the petriplate with different dilutions of 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6} for all the 5 samples. Average of triplicate plates were calculated with cfu/ml. Sample III showed highest bacterial count ranging from 300 cfu/ml to 315 cfu/ml, and is shown in Table II. This study was in line with the by Aydin in 2007 to assess the presence

of Gram-negative bacteria in groundwater intended for aquaculture farming. 15 species of bacteria related to 11 genera have been secluded from 100 samples of groundwater.

In ground water intended for aquaculture farming the number of total coliform bacteria and thermotolerant coliforms should not exceed 1000 cfu/100 ml and 10 cfu/100 ml limits respectively in accordance with International Standards [13]. According to [14] total coliforms, thermotolerant coliforms, *E. coli*, *Enterococcus spp. Salmonella* sp., *Staphylococcus* spp. and *P. aeruginosa* were detected in 25%, 17.5%, 15%, 47.5%, 15%, 27.5% and 15% of the groundwater samples, respectively.

Table II: Total Bacterial Count (cfu/ml)

Sample	Dilution	Total Bacterial Count (cfu/ml)			
		Plate 1 cfu/ml	Plate 2 cfu/ml	Plate 3 cfu/ml	Average cfu/ml
1	10^{-3}	111	123	119	117.6
	10^{-4}	108	102	107	105.6
	10^{-5}	99	98	93	96.6
	10^{-6}	87	91	90	91.3
II	10^{-3}	95	94	91	93.3
	10^{-4}	91	88	82	87
	10^{-5}	87	78	80	81.6
	10^{-6}	81	72	77	76.6
III	10^{-3}	317	316	313	315.3
	10^{-4}	309	307	309	308.3
	10^{-5}	305	306	305	305.3
	10^{-6}	301	300	300	300.3
IV	10^{-3}	95	93	90	92.6
	10^{-4}	90	82	80	84
	10^{-5}	85	74	80	79.6
	10^{-6}	80	65	67	70.6
V	10^{-3}	54	56	50	53.3
	10^{-4}	51	44	41	45.3
	10^{-5}	49	38	33	40
	10^{-6}	44	33	24	33.6

3.2 Results of Growth Curve

Among the 5 samples, sample 3 is found to be polluted with *E. coli*. Hence, growth curve was studied in the sample III with different incubation time at the optical density of 600nm.The results were shown in Table III and Graph I.

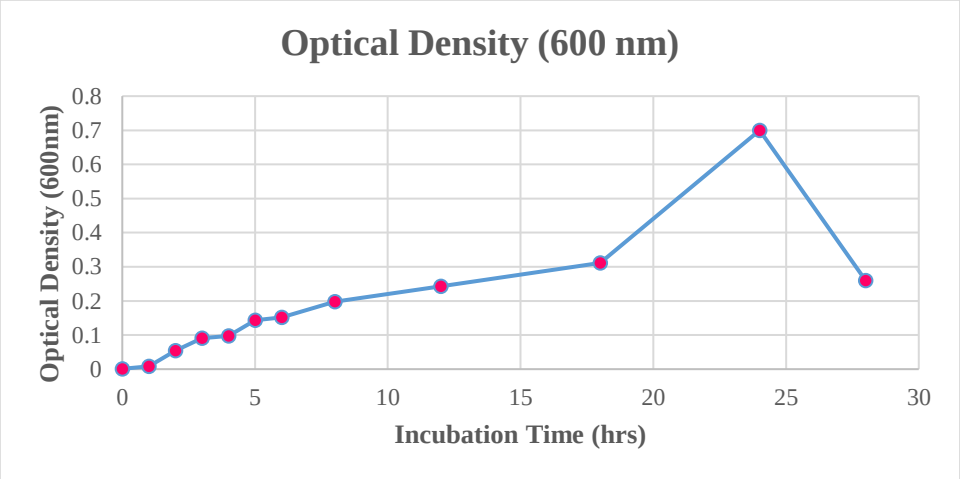
The present study has good correlation with Kalpan *et al*. [15] that the bacterial growth rates on PET surfaces and also in the nutrient broth were 1.09 and 2.61 h^{-1}, respectively. Hence,

the doubling times τ was 38 min and 16 min, respectively. The possibilities may be that the bacteria can replicate more in the solid-liquid interface, because half of the surface area will absorb more the nutrition from the surrounding; however, in the same time, they have to work more by using their appendages and remain constant in the surface. The other reason could be the unfavourable detachment of the daughter cells again into the particular medium, that ensures the reduction of bacterial count.

Table III: Results of growth curve in different incubation time

Sample Number	Incubation Time (hrs)	Optical Density (600 nm)
III	0	0.001
	1	0.008
	2	0.054
	3	0.091
	4	0.097
	5	0.143
	6	0.152
	8	0.198
	12	0.243
	18	0.311
	24	0.7
	28	0.26

Graph I: Graphical Representation for Growth Curve



3.3 Growth Curve of *E. coli*

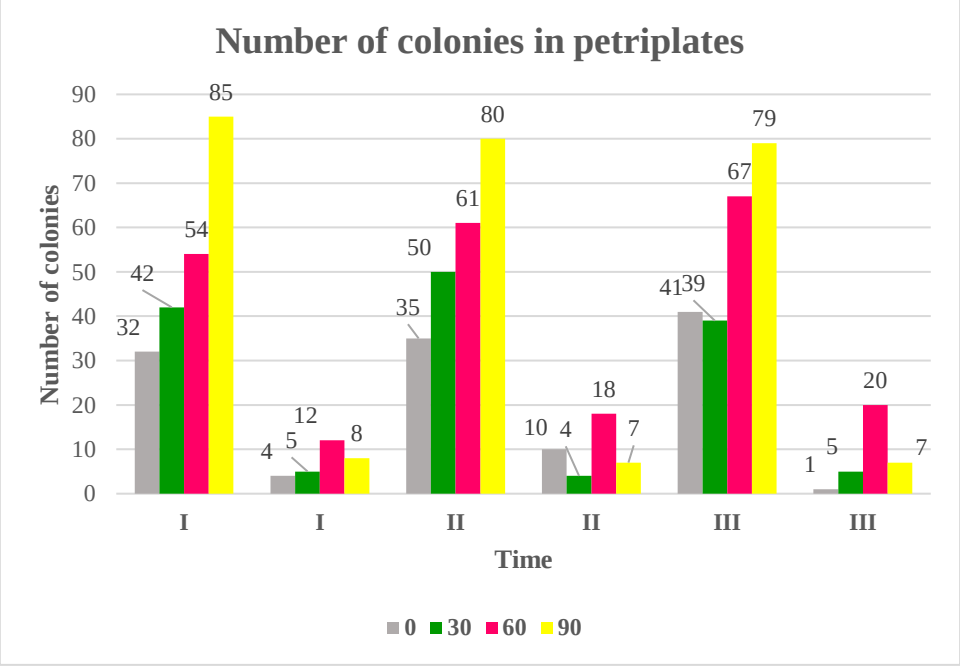
Table IV clearly depict the results of increasing growth of *E. coli* in a Nutrient broth with 10^{-5} and 10^{-6} dilutions. At 0 second, the average colony is noted as $3.6x10^{6}$, whereas in 30 seconds, the average colony is $4.6x10^{6}$, in 60 seconds, the average colony is approximately

$6.2x10^6$ and in 90 seconds, the average colony is $7.2x10^6$. Graph II also describes the results of growth curve at 10^{-5} and 10^{-6} dilutions.

Table IV: Results of Growth Curve of *E. coli* Grown at 37°C on Nutrient Broth (1 ml plated)

Time (sec)	Dilution Plated	Number of colonies in 3 petriplates	Average cfu / ml
0	10^{-5}	32, 35, 41	$3.6x10^6$
	10^{-6}	4, 10, 1	
30	10^{-5}	42, 50, 39	$4.6x10^6$
	10^{-6}	5, 4, 5	
60	10^{-5}	54, 61, 67	$6.2x10^6$
	10^{-6}	12, 18, 20	
90	10^{-5}	85, 80, 79	$7.2x10^6$
	10^{-6}	8, 7, 7	

Graph II: Results of Growth Curve of *E. coli* Grown at 37°C on Nutrient Broth (1 ml plated) at $10^{-5}$and 10^{-6} Dilution in Various Duration:



3.4 Identification of Bacteria

The microscopical, macroscopical and biochemical results revealed the presence of *Escherichia coli* and the results were shown in the Table V.

The identification of microorganisms is crucial for the researchers of medical and clinical microbiology. There are ultimately many standards for different methods adopted in the specific area for the identification of microorganisms [16].

Table V: Identification of bacteria

Characteristics	*E. coli*
Gram Staining	Negative
Shape (Cocci/Diplococci/Rods)	Rods
Motility (Motile / Non-Motile)	Motile
Capsule (Capsulated/Non-Capsulated)	Variable
Spore (Sporing/Non-Sporing)	Non-Sporing
Flagella (Flagellated/Non-Flagellated)	Flagellated
Catalase	Positive (+ve)
Oxidase	Negative (-ve)
Methyl Red Test	Positive (+ve)
Voges Proskauer Test	Negative (-ve)
Oxidative/Fermentative Test	Fermentative
Indole Production Test	Positive (+ve)
Citrate Utilization Test	Negative (-ve)
Urease Test	Negative (-ve)
Nitrate Reduction Test	Positive (+ve)
H_2S Production Test	Negative (-ve)
Gas Production Test	Positive (+ve)
TSI test	Positive (+ve)
EMB Agar	Metallic Sheen Colonies
Blood Agar	No Hemolysis
MacConkey Agar	Pink Colour Colonies

Table VI and Graph III depict the results for MPN, by comparing with MPN chart. Sample number 1, 2 and 5 showed the MPN index value of < 2, which is the nominal value

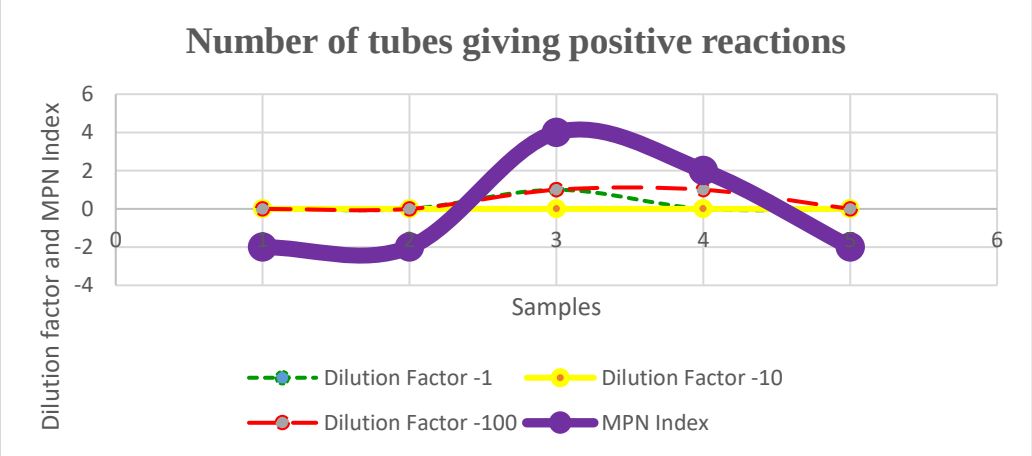
and not contaminated. Sample number 4 showed the MPN index value of 2, which is considered to be slightly contaminated but not so harmful. But sample number 3 has the MPN index value of 4, which is contaminated and hence that water cannot be used by the public.

Among the 10 water samples, that are unsatisfactory for drinking, 7 samples showed an MPN index of more than 1600/100 ml of water tested, whereas only 2 among the 5 corporation supplied water showed such high MPN index. This shows that mineral water being attested for its sterility is more unreliable than corporation water. In Pakistan, MPN count of coliforms/ 100ml, was 2.2-16 for tube well water samples, 1.1 - > 23 for water from distribution network and 2.2 to >23 for stored tank water 10. According to a study done by Murugesan *et al* in 2015, 70% of water supplied in bubble top cans in Chennai are within exceptional boundary of WHO11 whereas in our study 90 % (10/11) of water supplied in 20 litre bubble top cans (mineral water) are unsatisfactory for drinking. Out of ten well water sample processed in Kottayam district of Kerala state, 3 grew *Salmonella typhi* and six grew *Vibrio cholera* [Gopinath *et al*., 2012].

Table VI: MPN Index Value of Different Samples

Sample No.	Number of tubes giving positive reactions			MPN Index
	Dilution Factor -1	Dilution Factor -10	Dilution Factor -100	
1	0	0	0	< 2
2	0	0	0	< 2
3	1	0	1	4
4	0	0	1	2
5	0	0	0	< 2

Graph III: MPN Index Value of Different Samples



4. Conclusion

The present investigation recommends that minimum borehole depth of 50 m and surface distance from septic tank of 30 m is necessary. Therefore, it is advised that for borehole water to be potable it should be treated by chlorination or other treatment method suitable for public health. The quality of the well water could be enhanced by adopting proper waste disposal, water drains, managing sewage spills and also avoiding the open area defecation. The drinking water sources must be tested regularly and constantly to detect the microbiological quality, in order to avoid enteric diseases. A permanent monitoring network should be developed at least once in a year to check the microbial communities. People should be educated about the spread of water borne diseases and its control measures.

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