RESEARCH PAPER:

Seasonal changes in coliform contamination of potable ground water sources in Thiruvananthapuram, Kerala, India

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SUMMARY

In order to verify the level of microbial contamination in potable well water sources of Thiruvananthapuram city and their suitability for drinking purpose, the present study was conducted. The study area *i.e.* Thiruvananthapuram Corporation with 81 wards was classified into different zones by combining nearby wards. Samples were collected from dug wells from each zone during summer and monsoon seasons of two consecutive years *viz.*, 2006 and 2007. Samples were analysed for total coliforms and fecal coliforms. The samples which showed nil values for TC using MPN method were analysed by PCR . The number of coliforms, both total and fecal were much higher than the permissible limits in most cases. Most of the wells which had higher level of contamination were shallow, which could be the reason for ease of contamination. The hot spot of FC contamination was zone no. 4. The results throw light on the degree of microbial contamination in potable water sources, the seasonal changes as well as hot spots of contamination, which can be useful for further remedial and suitable precautionary measures to prevent outbreak of water borne diseases.

Key words :
Coliform contamination; Total coliforms (TC), Fecal coliforms (FC), Most probable number (MPN), Polymerase chain reaction (PCR)

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Drinking water safety is a worldwide concern. Drinking water may be contaminated by added discharges from human or animal intestines. Diseases caused through contaminated water and poor hygiene practices are leading cause of death among children worldwide (WHO, 2003). Biological hazard in the form of pathogenic bacteria, viruses etc. are responsible for a major portion of diarrhea, morbidity, gastro-intestinal disorder, cholera etc. in most developing countries.

Water contaminated with human feces are generally regarded as a greater risk to human health, as they are more likely to contain human-specific enteric pathogens, including *Salmonella enterica* serovar. *typhi*, *Shigella* spp., hepatitis A virus, and Norwalk-group viruses. Animals can also serve as reservoirs for a variety of enteric pathogens (e.g., various serotypes of *Salmonella*, *Escherichia coli*, and *Cryptosporidium* spp.) Ground water is generally less susceptible to contamination and pollution as compared to surface water bodies. But in India, where ground water is used extensively for irrigation and industrial purposes, a variety of land and water based human activities are causing pollution of this precious resource. Laluraj *et al.* (2005) reported a high incidence of open defecation in coastal parts of Kerala. Water has played a significant role in the transmission of human diseases and the indicator microorganisms have been used to suggest the presence of pathogens (Peeler *et al.*, 2006). Out breaks of a series of water borne diseases were reported from thickly populated areas in recent years especially during the rainy seasons. A survey conducted by Royee (2004) showed that 56% of households in Kollam Municipality discarded child feces in open places. In this context, the present study was aimed to find the source of microbial contamination in well water sources of Thiruvananthapuram Corporation in order to create awareness among the public about the safety of their drinking water.

MATERIALS AND METHODS

The study was intended to analyse the possibility of presence of pathogens through the analysis of indicators in the drinking water sources of Thiruvananthapuram Corporation. For the collection of water samples, the Corporation area which consists of 81 wards were grouped into 15 zones (Table 1) and 3 samples were collected from each zone based on the availability of wells for two consecutive years *viz.*, 2006 and 2007 during summer and monsoon seasons. Sampling was done

Table 1 : Well water sampling zones

Zone No.	Ward No.	Wards
1	1	Pallithura
2	2,3	Kulathoor, Attipra,
3	4,5	Kuzhivila, Cheruvakkal
4	6,14	Akkulam, Anamugham
5	81	Pound Kadavu
6	7,8	Ulloor, Pongummoodu
7	11,19,20,24	Muttada, Kuravankonam, Nalamchira, Nanthancodu
8	52,53,54,55	Nemom, Ponnumangalam, Melamcode, Pappanamcode
9	79,80	Karikkakom, Vettucaud
10	78	Sangumugham
11	70,71,72,61	Beemapalli, Valiathura, Sreevaraham, Kamaleswaram
12	63,64,65	Ambalathara, Thiruvallam, Punchakkarai
13	47,48,56,57	Arannoor, Karamana, Nedumangadu. Kalady
14	49,50,51	Mudavanmugal, Thrikkannapu-Ram, Estate
15	9,10	Edavacode, Mannanthala

according to Theroux *et al*. (1999) and the contamination status was determined by analyzing the presence of indicator organisms such as total coliforms (TC) and fecal coliforms (FC) both by conventional MPN method (WHO 1997). Those water samples showing negative results (0 values) in MPN technique were analysed by PCR (Eppendorf Master Cycler Personal) for the presence of pathogens and indicators. The PCR conditions were standardized using *Escherichia coli* cultures. The PCR conditions are given in Table 2.

Table 2 : Standardiszd PCR conditions

Step	Temperature	Time
1	95ºC	4 min
2	95ºC	1 min
3	50ºC	1 min
4	72ºC	2 min
5	Go to	Cycle 1
6	95ºC	10 min
7	Hold at 4ºC	-

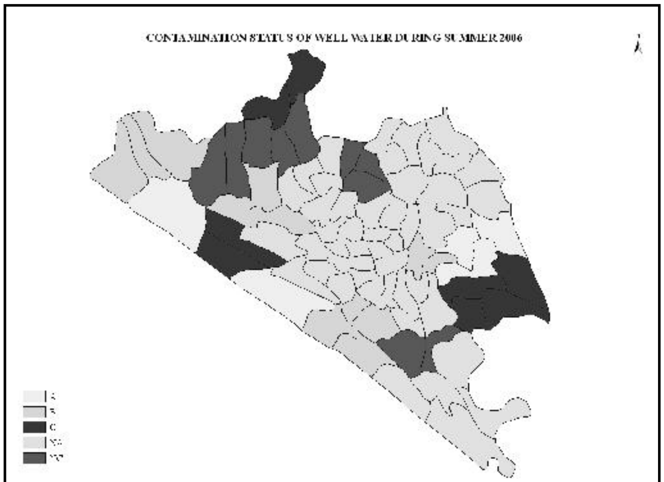
RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Total coliforms (TC):

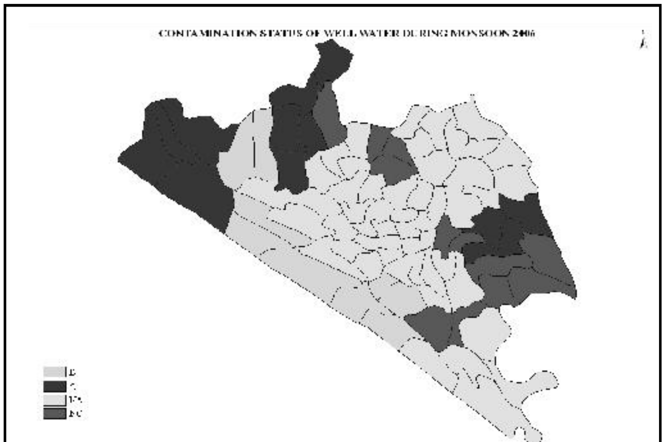
The Most probable number (MPN) of total coliforms (TC) during the summer of 2006 ranged from 0 to 3450. The highest level of contamination was observed in zone No. 8. During monsoon of the same year, the values ranged from 0 to 4099. The highest value was observed in zone No. 15.

Based on the number of coliforms observed, the level of contamination was divided into four categories *viz*., NC (0 cells/100mL), A (1-100 cells/100mL), B(101-1000 cells/100mL) and C(>1000 cells/100mL). 43.9% of the samples analysed during summer of 2006 belonged to NC category, 17.1% belonged to A, 24.4% belonged to B and 14.6% belonged to C. Contamination status of the wards can be seen in Fig. 1. During monsoon, 42.5% of samples fell in NC, 2.5% in A, 12.5% in B and 42.5%in C. The status of wards based on contamination levels during summer is shown in Fig. 1 and during monsoon in Fig.2



NC- 0 cells/100mL, A= 1-100 cells/100mL, B= 101-1000 cells/ 100mL, C= >1000 cells/100mL, NA= samples not available

Fig. 1 : Contamination status of zones during summer 2006



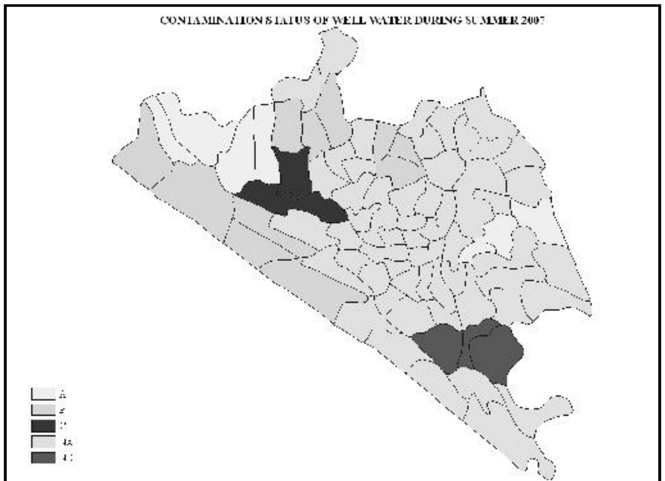
NC- 0 cells/100mL, A= 1-100 cells/100mL, B= 101-1000 cells/ 100mL, C= >1000 cells/100mL, NA= samples not available

Fig. 2 : Contamination status of zones during mansoon 2006

In the second year of study, *i.e*. 2007 the MPN of TC ranged between 0 and 1750 during summer. The highest value was observed in zone no. 4. During monsoon the values ranged between 0 and 1100, the highest value being recorded in zone No. 1

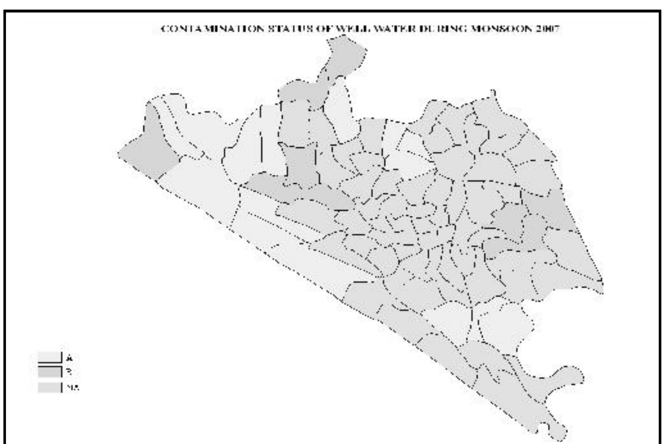
Based on the observations, during summer, 42.4% of the wards were grouped under NC; 18.2% under A; 30.3% under Band 9.1% under C. During monsoon, 39.4% were grouped under NC; 42.4% under A; 15.2% under B and 3.0% under C. The contamination level of different wards can be seen in Fig. 3 and 4 for summer and monsoon, respectively. The seasonal variation in TC for the two years is shown in Fig. 7.

Statistical analysis of the values showed that there was no significant variation in contamination during two



NC- 0 cells/100mL, A= 1-100 cells/100mL, B= 101-1000 cells/100mL, C= >1000 cells/100mL, NA= samples not available

Fig. 3 : Contamination status of zones during summer 2007



NC- 0 cells/100mL, A= 1-100 cells/100mL, B= 101-1000 cells/100mL, C= >1000 cells/100mL, NA= samples not available

Fig. 4 : Contamination status of zones during summer 2007

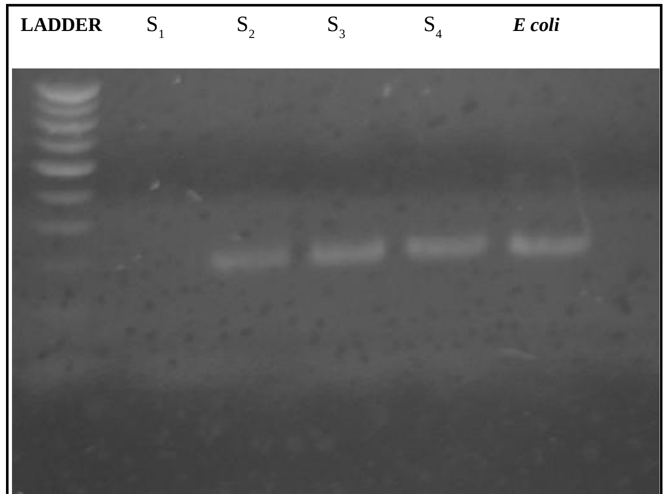


Fig. 5 : Amplified PCR products

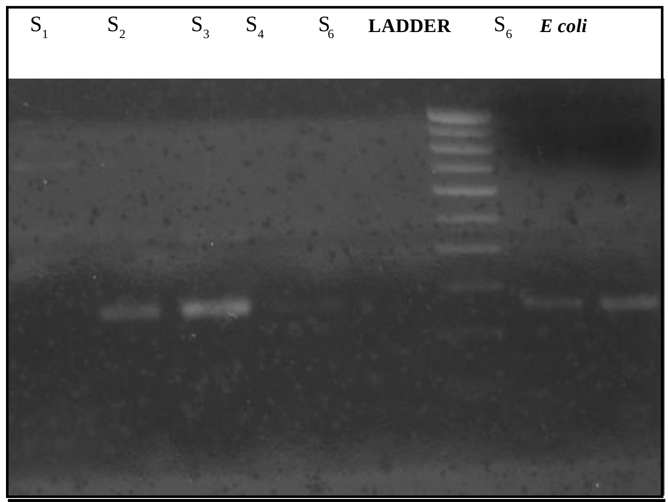


Fig. 6 : Amplified PCR products

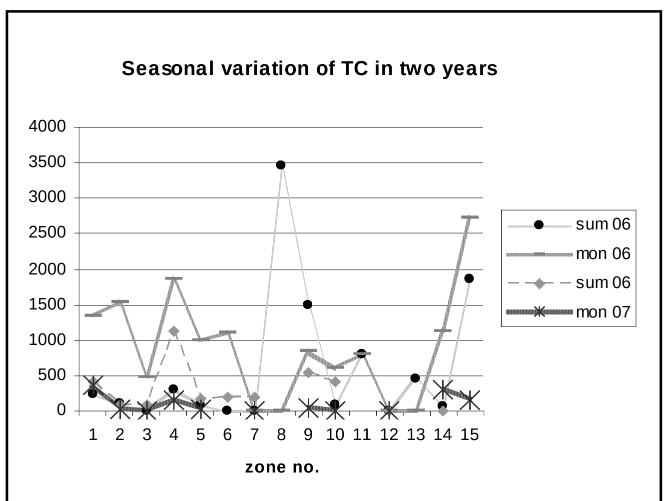


Fig. 7 : Seasonal variation in the values of TC in the two years

years. Seasonal variation was also found to be not significant (P value>0.01).

Though not statistically significant reduction in the values was observed during 2007 compared to 2006. During 2006, monsoon showed highest values. Contrastingly during 2007, summer has maximum value of TC. Normally the value should increase during rainy season. But the down pour was heavy and the period of rain was long. Hence, dilution is thought to have played a role in the decrease in contamination after an increase from that in summer season. Moreover, the increase in water temperature during summer, together with available nutrients etc. might have encouraged growth of bacteria (Geldreish, 1978).

Fecal coliforms (FC):

The analysis of MPN of FC during summer of 2006 showed values between 0 and1750 with zone No. 8 showing the maximum value. During monsoon, the values ranged between 0 and 1570 with zone No. 1 showing maximum value. 51.2% of samples during summer and 435 of samples during monsoon did not show the presence of fecal contamination. The values were found to be almost corresponding to the values of TC.

In the year 2007, the MPN of FC during summer ranged between 0 and 280. Maximum count was observed in zone no. 4. During monsoon the values ranged between 0 and 240. The highest value was observed in zone No. 14. Although the number of fecal coliforms observed was higher during summer, contamination was found to be more spread during monsoon. The seasonal variation of FC in the two years is given in the Fig. 8.

A statistically significant variation in the values of FC was observed between summer 2006 and monsoon 2006 (P=0.032, 5% level), between monsoon 2006 and summer 2007 (P= 0.007, 1% level) and between monsoon 2006 and monsoon 2007 (P=0.023, 5% level). Variation between two years was also found to be significant (P=0.033, 5% level).

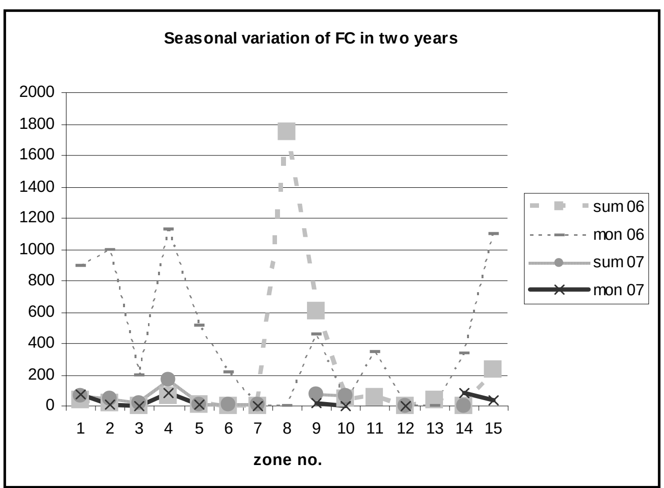


Fig. 8 : Seasonal variation in the values of FC in the two years

Polymerase chain reaction (PCR):

In the summer collection of 2006, 43 samples showed nil result by the conventional MPN technique. Of these, 7 gave positive result by PCR. During monsoon, of the 19 samples which showed negative result by the conventional method of detection of total coliforms, 4 gave positive result by PCR technique. Of the 23 samples which were found to be negative during summer of 2007, 5 were found to give positive result in the PCR method of detection of coliforms. Of the 27 samples analysed for PCR in monsoon, 2 were found to be positive. The results of PCR amplification can be observed in Fig. 5 and 6.

Analysis of coliform bacteria in wells of Thiruvananthapuram during summer and monsoon seasons of 2006 and 2007 revealed that during monsoon 2007, contamination was more spread among the different zones than other periods of collection. However, the highest values of TC in dug wells was observed during monsoon 2006 (4099) followed by summer 2006 (3450). A reduction in MPN of TC was observed in dug well samples from 2006 to 2007 even though it was not statistically significant (P= 0.07). Most of the wells which had higher level of contamination were shallow, which could be the reason for ease of contamination. The hot spot of FC contamination in well water was zone 4 (Akkulam and Anamugham wards). Srikantaswamy *et al*. (2008) reported that the quality of drinking water supply of Mysore varied from moderate to high level of contamination which stands along with the observation of this study.

Although water is not a medium for pathogenic growth, it is a means of transmission of the pathogen to the place where the individual is able to consume and therefore starts the out break of disease. To reduce the incidence and prevalence of water borne diseases, improvements in the availability, quantity and quality of water, improved sanitation, and general personal and environmental hygiene is required. Frequent monitoring of drinking water sources is a pre-requisite for access to safe drinking water by the public especially in less developed countries like India. Suitable awareness programme must be followed to educate the public in good hygienic practices include safe disposal of human and animal excrement.

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