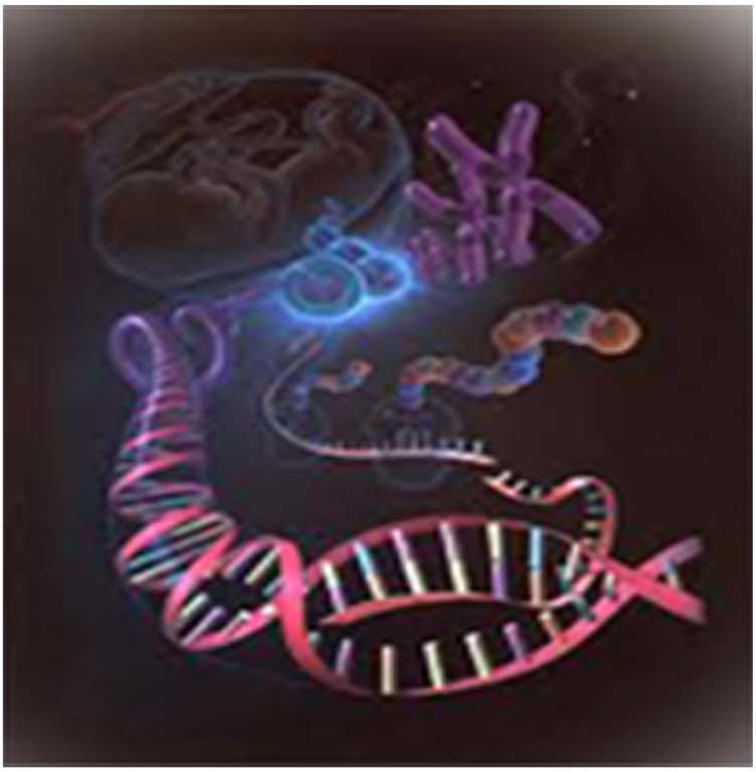
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 3, July 2013

International Journal of Life Sciences Biotechnology and Pharma Research



www.ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 3, July 2013
© 2013 IJLBPR. All Rights Reserved

Case Study

BACTERIOLOGICAL CONTAMINATION OF GROUNDWATER DUE TO ONSITE SANITATION PROBLEMS IN KERALA STATE: A CASE STUDY

P S Harikumar[1]* and K Madhava Chandran[2]

**Corresponding Author: P S Harikumar,* ✉ *drpshari@yahoo.co.in*

An investigation was carried out to determine bacterial contamination of groundwater and its causative factors in selected areas of Kozhikode district in Kerala State, India. Multiple tube fermentation technique was used to determine Total coli forms (TC), Faecal coli forms (FC) and *Escherichia coli*. As per FC: FS ratio, 55.5% of water samples were contaminated due to unscientific construction of latrines, 11.1% by an animal source, while 33.3 % had mixed contamination from both sources. Further confirmation was done using nucleotides homology and phylogenetic analysis. One of the detected organisms was *Bacillus cereus*, contributed by human faeces. The nine independent variables of the study contribute to 53.9% of the variance observed in the total water quality score of the sample wells, with distance of latrine from the well and presence of *E. coli* exerting statistically significant influence. Results indicate that less than 25% of the wells with lining and less than 16% of the wells located more than 7.5 M from the latrine have the likelihood of presence of *E. coli*.

Keywords: Bacteriological contamination, Faecal coli forms, Safe distance

INTRODUCTION

Water and sanitation infrastructure of Kerala state in India is extensive and valuable. According to a recent survey by CWRDM, a research organization of the Govt. of Kerala, there are 67 lakh open wells. 80% of the population in Kerala is estimated to depend on groundwater for different uses. This number of wells in the state represents a massive investment of Rs. 4,500 cr. New investment in wells is being done through the Kerala Rural Water Supply and Sanitation Agency and Swaljaladhara programs that are facilitating the construction of community wells. Kerala also has the highest coverage of individual household latrines in India (Jithendran, 2003). Policies and programs must appropriately invest in getting safe potable water and awareness to limit the exposure of people to faecal pathogens and chemical contamination. Conditions for water

1 Water Quality Division, Centre for Water Resources Development & Management, Kozhikode, Kerala, India.
2 Water Management Division, Centre for Water Resources Development & Management, Kozhikode, Kerala, India.

supply in Kerala are pretty much typical of (sub) urban-rural areas of developing countries. According to the census data from 2011, Kerala holds a total population of 33.3 million, rendering a density of 859 persons per km². Majority of the population reside in the coastal belt, with high population density. These areas commonly have a well density of 200 wells/km^2. In rural and peri-urban areas, the access to safe latrines is low. A large majority of rural households, especially belonging to the poor and the vulnerable, are without latrines or latrines which are not constructed scientifically. The morbidity rates are high, especially with regard to diseases related to water and sanitation. More than 600,000 people annually contract some form of gastrointestinal illness and seek medical help. A study was conducted by CWRDM indicates that 70% of drinking water wells in Kerala have faecal contamination (Harikumar, 2009).

Contamination of groundwater wells can occur from both above and below the surface. Pollution of entire groundwater aquifers may occur from failing septic systems, manure and fertilizer applications, mining, or other land uses. Individual water supplies may also be contaminated around the exposed well casing (wellhead) from surface water flowing along the well casing and/or from a loose fitting or absent well cap that allows insects, animals or surface water to directly enter the well (Bryan *et al.*, 2009).

Sources of microbial contamination of groundwater include agricultural runoff, effluents from septic tank, sewage discharges, and infiltration of dissolved wild animal fecal matter. Poor well maintenance and construction (particularly of shallow dug wells) can increase the risk of bacteria and other harmful microorganisms getting into the water supply. The source of contamination is dependent on the type of settlement, population density, sanitation arrangements, and sanitation behavior (Lawrence *et al.*, 2001).

The greatest impact on pollution is by point source on-site sanitation systems, which include pit latrines, septic tanks, etc. Ground water derived from deep aquifers is generally of good bacteriological quality because vertical percolation of water through soil results in the removal of much of the microbial and organic pollution. Infiltration of the septic tank effluent into unsaturated soil makes the soil contaminated, with resulting ground water contamination (Mc Feters, 1986).

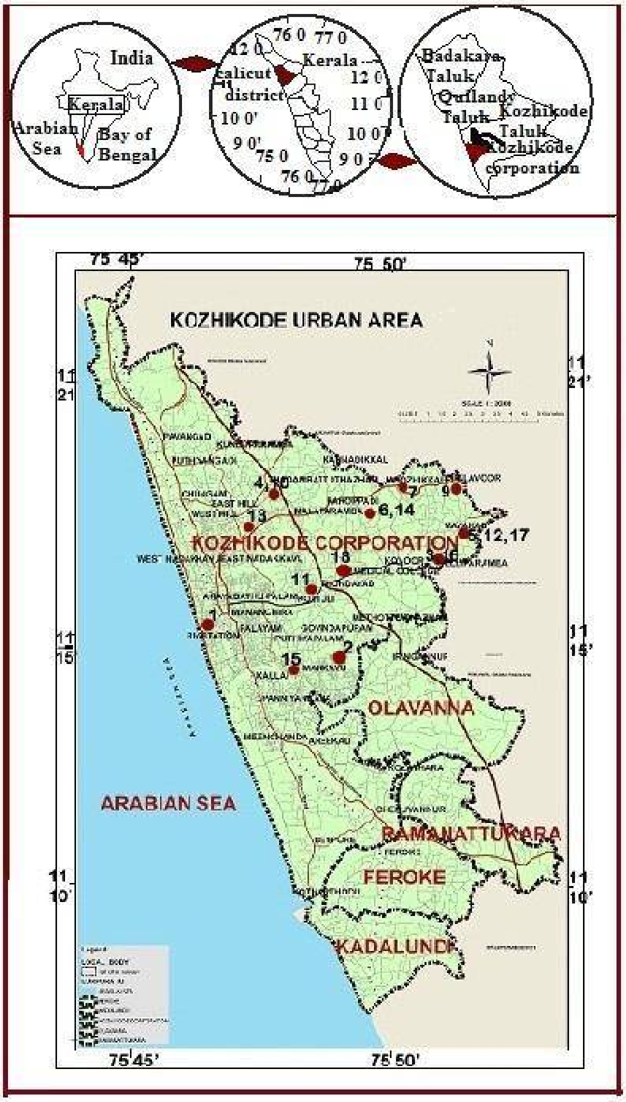
An investigation was carried out to assess the seasonal bacteriological contamination of selected ground water sources due to on-site sanitation problems in Calicut Corporation located in Kozhikode district in the State of Kerala in India, and to identify sources of contamination. The water quality score of groundwater samples were worked out and the relationship between the score and various contributing factors (variables) were established through statistical tests.

MATERIALS AND METHODS

Location of Study

The water samples and soil samples for study was collected from different locations in the Calicut Corporation. Calicut Corporation is situated in Kozhikode district in the northern part of Kerala State, India, at latitude 11°21 N and longitude 72°22 E (Figure 1). Population of Calicut city is 432,097 as per official census report for 2011. The population density of the Calicut Corporation is 5,078/km^2. Samples were collected from 24 dug wells distributed at various locations in the

Figure 1: Map of Calicut Corporation Showing the Sampling Sites



Sample code	Location
1	Beach road
2	Pokkunnu
3	Medical college
4	karaparamba
5	Mayanad
6	Chevarambalam
7	merrikunnu
8	Jain Temple
9	Chelavur
10	Karaparamba
11	Kottuli
12	Mayanad
13	Eranjipalam
14	Chevarambalam
15	Chalapuram
16	Medical college
17	Mayanad
18	Nellikode

city. Samples were repeatedly taken from six mostly contaminated sites based on preliminary sampling and analysis.

Water and Soil Sampling

Water samples were collected in sterile bacteriological bottles from 18 different sites of Calicut Corporation (Figure 1) seasonally viz; pre-monsoon (Feb-May), monsoon (June-Sep) and post-monsoon (Oct-Jan). Soil samples were collected from grossly contaminated sites at various distances from the latrines. Samples were also collected from a freshly dug pit. The pit was dug to a depth of 3 m with the help of an auger. The soils of Calicut Corporation have been classified into four groups based on their morphological features and physico-chemical properties. These are Coastal alluvium, Lateritic soil, Riverine alluvium and Brown hydro-morphic soil. The soil is immature, with high sand content, low water holding capacity, etc., and the pH value is less than 6.5 in most of the area.

Water and Soil Analysis

Most Probable Number (MPN) technique was used for the determination of total coli forms (TC), fecal coli forms (FC), and *Escherichia coli. Fecal Streptococcus* (FS) from the water samples were also analyzed during each season [pre-monsoon (Feb-May), monsoon (June-Sep) and post-monsoon (Oct-Jan)] so as to find the FC/FS ratio, which is a useful indicator for finding out the source of contamination. Bacterial identification using PCR based 16s rDNA was performed to identify the organism causing contamination.

The collected soil samples were serially diluted with sterile distilled water. The dilution used was in the range 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The MPN test was performed to identify the total coli forms, faecal) coli forms, and *Escherichia coli.* The MPN of the soils were carried out using 10^{-2} dilutions. A soil column experiment was conducted using a glass column of dimension 45 cm length and 2 cm inner diameter. Approximately 10 g of the soil samples were used to conduct the study. The soil was saturated with distilled water and the column was packed with saturated soil in sterile condition. A column height of 5 cm was maintained. This column was eluted with distilled water and samples were collected in regular intervals. The collected samples were analyzed for the total coli forms. The experiment continued until the fraction of eluted water did not give any bacterial count.

Simeonov *et al.* (2003) highlights the necessity and usefulness of multivariate statistical assessment of data bases to get better information about quality of water, the design of sampling and analytical protocols, and effective pollution control/management of water. The use of statistical models showing the relationship between water quality and contributing factors strengthen the conclusions that may be drawn from analysis of water quality and sanitary inspection data. Such approaches allow better interpretation of data and provide a more sound basis, from which to plan water and sanitation investments (Guy Howard *et al.,* 2003). Vida Rutkoviene *et al.* (2005) report that pollution of shallow well water is an indication of environmental pollution, and is especially susceptible to anthropogenic factors such as farming intensity, potential pollution sources in the sanitary zone and well protection from environmental pollution. High rates of morbidity and mortality are partly related to the bad quality of shallow well water. Based on multiple regression analysis of the data of 809 shallow wells from 35 villages of Kaunas District in Lithuania, they have reported that distance from the well to the outhouse, cow shed, manure clamp and kitchen garden, water level in the well and quality of well construction and protection are the factors exerting greatest influence on the pollution of the wells. The importance of such data in estimating the impact of anthropogenic factors on changes in environmental quality and forecasting the level of pollution are also reported in this study.

In the present study, taking into consideration the research work cited above, the relationship between various factors (considered as independent variables) and total water quality score of groundwater taken from wells (considered as the dependent variable) was determined using relevant multivariate statistical

This article can be downloaded from http://www.ijlbpr.com/currentissue.php

techniques. For this, scoring was adopted for different variables, as explained below

Dependent variable: The dependent variable of the study is total water quality score of groundwater. This is the summated score of the following water quality parameters, namely, color, turbidity, hardness, TDS, pH, *Escherichia coli*, nitrate nitrogen, phosphate phosphorus, chloride, iron, sodium, calcium and magnesium. A score of 1 was given for the presence of each of these parameters, and 2 for their absence in well water.

Independent variables: The independent variables included in this study and the scoring pattern adopted for them are shown in Table 1.

Table 1: Scoring Pattern Adopted for Independent Variables

S. No.	Variable	Details	Score
1	Type of well lining	Concrete	3
		Laterite	2
		Un-lined	1
2	Parapet height of well	Refer Note	1
3	Mode of water lifting from well	Pump	2
		Pulley	1
4	Use of fertilizers	Yes	1
		No	2
5	Use of PP chemicals	Yes	1
		No	2
6	Type of latrine	Septic Tank	4
		Pit type	3
		Leach type	2
		Open defecation	1
7	Latrine distance from well	Distance in m.	Refer Note 2 below the table
8	Cattle shed distance from well	Distance in m.	Refer Note 3 below the table
9	Presence of E.coli in well	Present	1
		Absent	2

Note:
1. Ideal parapet height of well considered is 0.75 m (score = 2). For every additional 1m height, additional score of 1 is allotted. Less than 0.75M (score = 1).
2. Ideal distance considered from latrine to well to is 7.5 m. (score = 2). For every additional 1 m distance, additional score of 1 is allotted. Less than 7.5 M (score = 1).
   As per the building rules of the Government of Kerala, India, the safe distance stipulated between latrine and open well is 7.5 m.
3. Ideal cattle shed distance considered from well is 7.5 M (score = 2). For every additional 1m distance, additional score of 1 is allotted. For no cattle shed, a score of 1 more than the maximum score observed in the sample under study is allotted.

RESULTS AND DISCUSSION

The results show that the groundwater samples of Calicut Corporation are bacteriologically contaminated. Observations revealed that the contaminated wells are mostly constructed very near or down-gradient from the latrines. This implies that the risk of contamination is very high. The bacteriological analysis of the water samples revealed that they are unsafe for drinking because of fecal contamination. The sampling locations, namely, Karaparamba, Nellikode, South Beach road, Pokkunnu, Cheleot road and Kottooli were found to be highly contaminated. The distribution of bacteria in the water samples collected during different seasons is shown in Figures 2 and 3. The level of contamination is high during monsoon season and all the samples showed the presence of *Escherichia coli*.

Figure 2: Seasonal Distribution of Total Coli Forms in Groundwater Sources of Calicut Corporation

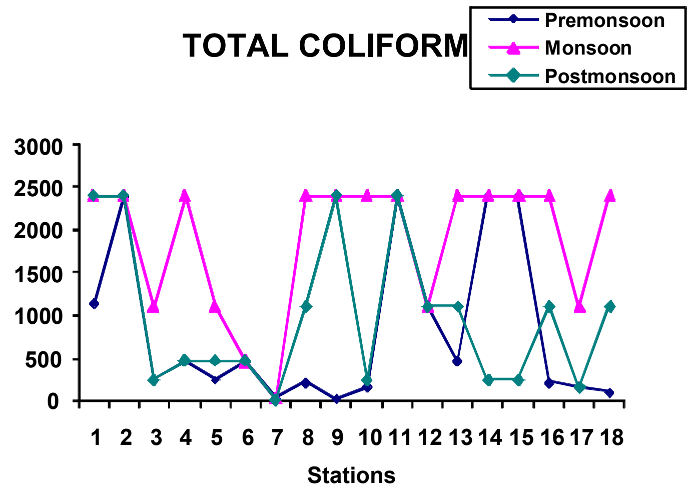
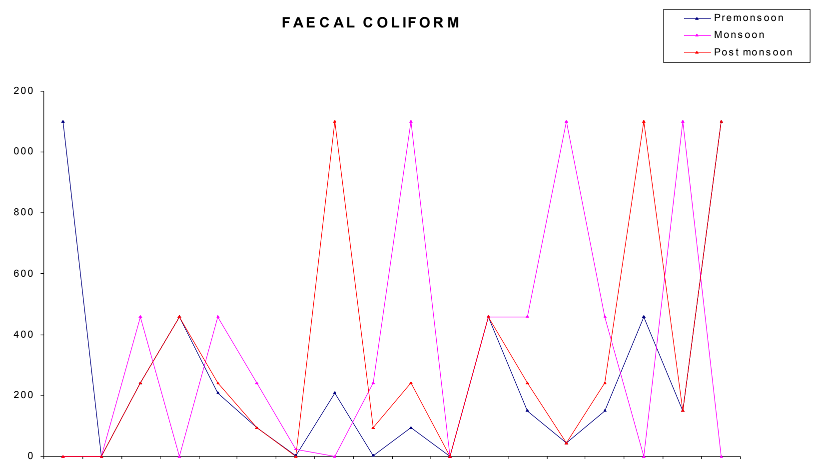


Figure 3: Seasonal Distribution of Fecal Coli Forms in Groundwater Sources of Calicut Corporation



In the 1950's and 1960's, the Fecal Coli form to Fecal Streptococcus (FC: FS) Ratio was used to identify the cause of contamination. The FC:FS ratio of human waste is greater than 4, while that of animal waste is greater than 0.7. But this has been questioned later (Cara Gleeson, 1997). In the present study, this ratio was used as a preliminary step to identify the locations prone to human contamination. This was further confirmed by PCR analysis of selected samples. The FC/FS ratio was calculated to find out the source of contamination (Coyne and Howell, 1994; Maria Csuros and Csaba Csuros, 1999). The results indicated that the ratio ranged from maximum of 52.3 to a minimum of 0.22. Of the nine samples collected, five samples reported value greater than 4. The ratio highlighted that the human faeces might be the main source of contamination, but in case of some samples, the source of contamination was found to be of both human and animal origin. The FC/FS ratio for domestic animal contamination is less than 1.0, whereas, the ratio for human contamination is more than 4.0. When ratios in the range of 1-2 are obtained, the most suitable interpretation is that the pollution is derived from both human and animal sources (Jacobs, 1991).

Based on nucleotides homology and phylogenetic analysis, the organism detected was *Bacillus cereus*. This is widely distributed in the environment and causes food borne diseases. The presence of this organism in water indicates faecal pollution because these organisms enter the environment through faeces of a man infected by diarrhoea.

Soil samples from highly contaminated areas were collected and subjected to bacteriological analysis. The results of analysis (MPN) using 10^{-2} dilution are given in Table 2. The results indicate a heavy load of bacteriological contamination. Total coli forms, faecal coli forms, faecal Streptococci, and *E. coli* were detected in all the samples.

Factors Influencing Water Quality of Wells

Table 3 shows the results of multiple linear regression analysis of nine independent variables on the dependent variable—water quality score of wells. The nine variables together explain 53.9% of the variance observed in the total water quality score of the sample wells, with the regression being highly significant. It can also be made out from the table that the distance of latrine from well and presence of *E. coli* are the two factors exerting significant influence on the water quality

Table 2: Bacteriological Analysis of Soil Samples

Parameter	Sample Number					
	2	18	4	1	9	11
Total coli forms MPN/100ml	≥2400	≥2400	≥2400	≥2400	≥2400	≥2400
Faecal coli forms MPN/100ml	≥2400	≥2400	≥2400	≥2400	≥2400	≥2400
E. coli	Present	Present	Present	Present	Present	Present
Faecal Streptococci	15	75	93	43	93	93
Faecal Streptococciplate count	3	11	6	3	7	3

Table 3: Multiple regression analysis of variables influencing water quality score

Variable	Regression Coefficient	t
Type of well lining	-0.469	-0.288
Parapet height of well	-0.408	-1.595
Mode of water lifting from well	-0.447	-1.094
Use of fertilizers	-0.104	-0.227
Use of PP chemicals	-0.357	-0.528
Type of latrine	-0.367	1.382
Latrine distance from well	1.295**	1.990
Cattle shed distance from well	-0.507	-0.965
Presence of E.coli in well	3.316*	6.099
R^2 = 0.539*		

Note: * Significant (0.00 probability); ** Significant (0.05 probability)

score of wells, with *E. coli* exerting a higher influence.

Linear regression analysis was also carried out to separately analyze the contribution of the factors, namely, latrine distance from well and presence of *E. coli* in the well on water quality. The result reveals that a high level of the variance (46.7%) in water quality score is explained by these two variables (Table 4). Further, it can be made out from Table 5 that presence of *E. coli* alone contributes to about 42% of variation in water quality. These findings confirm the profound influence of *E. coli* on the water quality status of the wells in this study. Hence, this is an important aspect to be considered, while planning proper sanitation measures to ensure water quality.

Presence of *E. coli* in water can be influenced by the distance of latrine as well as the distance of cattle shed from the well. However, since, the latter variable did not show significant influence in the multiple regression analysis, an attempt was made to establish the relationship between latrine distance from well and presence of *E. coli*. Table 6 shows a significant regression coefficient, with 78.7% of variance in presence of *E. coli* being explained by the distance of latrine from the well.

Hence, it may be inferred from this study that latrines located at a distance of less than 7.5 m from the wells is the primary factor contributing to the presence of *E. coli* in the wells. This is confirmed from chi-square test, which shows that latrine distance influences presence of *E. coil* in the wells (Table 7). It can also be made out from Table 7 that in the case of wells located more than 7.5 M from the latrine, only 11.8% of them show presence of *E. coli*, unlike in wells located less than 7.5 M, where, 46.2% show presence of *E. coli*. This is again confirmed through z test, where significant difference is observed in presence of *E. coli* between wells located less than 7.5 M and wells located more than 7.5 m from latrine (Table 7). In addition to latrine distance,

Table 4: Influence of Latrine Distance and *E. coli* on Water Quality Score

Variable	Regression Coefficient	*t*
Latrine distance from well	1.340**	2.075
Presence of E. coli in well	3.300*	6.393
R^2 = 0.539*		
Note: * Significant (0.00 probability); ** Significant (0.05 probability)		

Table 5: Influence of *E. coli* on Water Quality Score

Variable	Regression Coefficient	*t*
Presence of E. coli in well	3.532*	6.829
R^2 = 0.429*		
Note: * Significant (0.00 probability).		

Table 6: Influence of Latrine Distance on *E. coli*

Variable	Regression Coefficient	*t*
Latrine distance	-0.983*	-15.120
R^2 = 0.787*		
Note: * Significant (0.00 probability).		

Table 7: Distance of Latrine from Well and Presence of *E. coli*

Latrine Distance from well	*E. coli* Present in well		*E. coli* Absent in well		Total		Chi-square value	df	Sig.
	No.	%	No.	%	No.	%	8.04	1	0.01
>7.5 M	6	11.8	45	88.2	51	100			
<7.5 M	6	46.2	7	53.8	13	100			
Total	**12**	**18.8**	**52**	**81.2**	**64**	**100**			

Table 8: Test of Significance Of Presence Of *E. Coli*

No. of				*z*-Stat	No. of				*z*-Stat
Lined Wells	**Unlined wells**	**Wells in col. (a) having *E. coli***	**Wells in col. (b) having *E. coli***		**Wells located >7.5M from latrine**	**Wells located <7.5M from latrine**	**Wells in col. (f) having *E. coli***	**Wells in col. (g) having *E. coli***	
(a)	**(b)**	**(c)**	**(d)**	**(e)**	**(f)**	**(g)**	**(h)**	**(i)**	**(j)**
34	30	3	9	-2.17*	51	13	6	6	-2.84*

Note: *Significant at 0.05 probability

Table 9: Well lining and presence of *E. coli*

Latrine Distance from well	*E. coli* Present in well		*E. coli* Absent in well		Total		Chi-square value	df	Sig.
	No.	%	No.	%	No.	%	8.04	1	0.01
With lining	3	8.8	31	91.2	34	100			
Without lining	9	30.0	21	70.0	30	100			
Total	12	18.8	52	81.2	64	100			

well lining also showed a significant influence (z statistic) on the presence of *E. coli* in wells (Table 9). It can be made out from Table 9 that *E. coli* is there in 30% of un-lined wells, while only 8.8% of the lined wells show its presence.

Even though the type of latrine did not show any significant influence on water quality in the multiple regression analysis (Table 3), none of the households having presence of *E. coli* in the wells are found to have septic tank latrine. 91.7% of these houses have leach type latrine and 8.3% have pit latrine. However, in the case of households having wells, which do not show the presence of *E. coli*, all of them are having septic tank latrine.

In order to assess the risk or likelihood of the presence of *E. coli* relative to lining of wells and distance of latrine from the wells, odds ratio was worked out. Odds ratio indicates relative risk, as a ratio of event probabilities. The results are presented in Tables 10 and 11. The odds ratio of 0.226 in Table 10 indicates that less than one-fourth (25%) of the wells with lining have the risk/ likelihood of presence of *E. coli*, when compared

Table 10: Odds Ratio of the Presence of *E. Coli* Relative to its Absence Based on Lining of Wells

E. coli	Frequency of occurrence of *E. coli*		Total	Odds Ratio
	in lined wells	in un-lined wells		
Present	3	9	12	0.226
Absent	31	21	52	

Table 11: Odds Ratio of the Presence of *E. coli* Relative To Its Absence Based On Location Of Wells From The Latrine

E. coli	Frequency of occurrence of *E. coli*		Total	Odds Ratio
	in wells located >7.5 M from latrine	in wells located <7.5 M from latrine		
Present	3	9	12	0.226
Absent	31	21	52	

This article can be downloaded from http://www.ijlbpr.com/currentissue.php

to wells without lining. Similarly, with an odds ratio of 0.156, less than 16% of the wells located more than 7.5 M from the latrine have the risk/likelihood of presence of *E. coli*, when compared to wells located less than 7.5 M from latrine (Table 11).

Hence, based on the statistical analyses on the influence of various factors on water quality of the wells, it may be inferred that lining of wells (with concrete or laterite) and location of latrines at a distance of more than 7.5 M from the wells can help to reduce the occurrence of *E. coli* in the water samples. Similar findings have also been reported by Charles Muruka *et al*. (2012).

Water quality scores of the wells were also categorized based on mean plus and minus one Standard Deviation (SD) value. Since there are no wells having total water quality score more than mean plus one SD, the wells have been classified into 'less' water quality score (score less than mean minus one SD) and 'more' score (score between mean minus one SD and mean plus one SD) categories.

With regard to the wells having 'less' water quality score, 60% of them were found to have less than 0.75 M parapet height, none of them have septic tank latrine, 40% of the wells have only laterite lining, while 10% wells are un-lined. Also, 60% of the wells are located at distance of less than 7.5 M from the latrine and 30% of them show presence of *E. coli*. For those wells having 'more' water quality score, 53.7% of them have concrete well lining, about 20% of the households possess septic tanks, 83% of the wells are located at distance of 7.5 M and more from the latrine, and only 9.3% of the wells show presence of *E. coli*.

CONCLUSION

Majority of the water samples collected from open wells of Calicut Corporation area in Kozhikode district, Kerala State, India were found to be bacteriologically contaminated and the contamination is mainly faecal in origin. *Escherichia coli* was present in majority of the samples. High level of *Faecal streptococcus* is present in the wells of the polluted areas. The normal habitat of *Faecal streptococcus* is the intestine tract of men and animals. The FC/FS ratio implies that the human sources of contamination are more significant than animal sources, indicating problems due to on-site sanitation. The application of FC/FS ratio is limited due to the variable survival rates of *Faecal streptococcus* species. Based on the nucleotides homology and phylogenetic analysis, the organism detected was *Bacillus cereus*, which also indicates contamination due to human origin. Analysis of soil samples indicate high pollution load.

The score for water quality of wells was worked out as the summated score of the parameters, namely, color, turbidity, hardness, TDS, pH, *Escherichia coli,* nitrate nitrogen, phosphate phosphorus, chloride, iron, sodium, calcium and magnesium. Multiple linear regression analysis revealed that the variables, namely, type of well lining, parapet height of well, mode of water lifting from well, type of latrine, distance of latrine from well, distance of cattle shed from well, presence of *E. coli* in well, use of fertilizers and PP chemicals together explain 53.9% of the variance observed in the total water quality score of the sample wells, with the regression equation being highly significant. Analysis of the contribution of distance of latrine from well and *E. coli* on water quality showed that about 47% of the variance in

This article can be downloaded from http://www.ijlbpr.com/currentissue.php

water quality score is explained by these two variables, with *E. coli* alone contributing to about 42% of the variation.

Linear regression analysis also revealed that distance of latrine from the well exerts a significant influence on presence of *E. coli*, with 78.7% of variance in presence of *E. coli* being explained by it. This was also confirmed from the chi-square and *z*-test results with these parameters. In the case of wells located more than 7.5 M from the latrine, only 11.8% of them show presence of *E. coli*, unlike in wells located less than 7.5 M, where, 46.2% show presence of *E. coli*. Based on odds ratio, it was evident that the risk/likelihood of wells with lining to have presence of *E. coli* is less than 25%, when compared to wells without lining. Similarly, the likelihood of wells located more than 7.5 M from latrine to have presence of *E. coli* is less than 16%, when compared to wells located less than 7.5 M from latrine.

Periodical monitoring of water quality of wells and identification of the factors contributing to contamination/pollution will help in developing appropriate source control measures for reducing pollution of groundwater sources.

REFERENCES

1. Bryan R. Swistock, M.S, Stephanie Clemens, M.S. and William E Sharpe (2009), "Drinking Water Quality in Rural Pennsylvania and the Effect of Management Practices", at: http://www.rural.palegislature.us/drinking_water_quality.pdf (Accessed on 7 July 2012).
2. Cara Gleeson N F (1997), *The Coliform Index and Waterborne Disease*, Taylor and Francis Publishers, New York.
3. Charles Muruka, Francis Adeniyi Fagbamigbe, Andrew Muruka, John Njuguna, David Odhiambo Otieno, Jack Onyando, Zulekha Saidi Wanjiku and Zablon Onyango (2012), "The Relationship Between Bacteriological Qualities Of Dug-wells & Pit Latrine Siting In An Unplanned Peri-urban Settlement: A Case Study Of Langas – Eldoret Municipality, Western Kenya", *Public Health Research*, Vol. 2, No. 2, pp. 32-36.
4. Coyne M S and Howell J M (1994), "The Fecal Coliform/Fecal Streptococci Ratio (FC/FS) and Water Quality in the Bluegrass Region of Kentucky", *Soil Science News & Views*, Vol. 15, No. 9.
5. Guy Howard, Stephen Pedley, Mike Barrett, Mai Nalubega and Kali Johal (2003), " Risk Factors Contributing to Micro Bacteriological Contamination of Shallow Groundwater in Kampala, Uganda", *Water Res.*, Vol. 37, No. 14, pp. 3421-3429.
6. Harikumar P S (2009) ",Water Quality Status of Kerala With Special Reference To Drinking Water. 2009", *Proceedings of Kerala Environment Congress,* pp. 90-105.
7. Jacobs J L and Ellis J B (1991), "Bacterial Water Quality In Urban Receiving Waters", *Water Science and Technology*, Vol. 24, pp. 113-116.
8. Jithendran V (2003), "Road Map To Total Sanitation", *Kerala calling*. October, pp. 8-10.
9. Lawrence A R, Macdonald D M J, Howard A G, Barrett M H, Pedley S, Ahmed K M and Nalubega M (2001), "Guidelines for Assessing the Risk to Groundwater from On-Site Sanitation", *British Geological Survey Commissioned Report*, Vol. 142, pp. 1-104.

10. Maria Csuros and Csaba Csuros (1999), *Microbiological Examination of Water and Wastewater*, CRC Press New York.

11. McFeters, Mark W LeChevallier, Ajaib Singh and Joyce S Kippin (1986), "Health Significance and Occurrence of Injured Bacteria in Drinking Water", *Water Science & Technology,* Vol. 18, No. 10, pp. 227-231.

This article can be downloaded from http://www.ijlbpr.com/currentissue.php

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137
03
9 772250 313001