



**Physicochemical and Microbiological analysis of well water samples collected from North of Punnapra village, Alappuzha district, Kerala state, India.**

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**Abstract**

The microbial flora of water plays a major role in determining the quality of potable water. The present study focused on the physicochemical and microbiological analysis of well water samples from Punnapara village, Cherthala, Alappuzha District, Kerala State, India. The first and the foremost step was to assess the organoleptic character of water samples and all the water samples showed acceptable quality. The pH of the water samples ranged from 6 to 7.14 and temperature ranged from 27.4 to 27.5 °C. The electrical conductivity ranged from 238 µs to 625 µs. The chemical parameters such as alkalinity and hardness ranged from 0 to 50 and 6 ppm to 13.3 ppm respectively. The Biological Oxygen Demand (BOD) of water samples ranged from 0.3 mg/l to 1 mg/l and Chemical Oxygen Demand (COD) ranged from 136 mg/L to 230.4 mg/L. Heterophillic bacterial, fungal and actinomycetes counts were analysed. Most probable number (MPN) technique was performed for all the water samples collected and all of them were found unfit for consumption. The presence of waterborne pathogens in well water samples were analysed by plating them on the specific agar media. Several pathogens such as *E. coli*, *Salmonella typhi*, *Enterococcus faecalis* and different *Vibrio* species were detected in water samples.

**Keywords:** microbial flora, physicochemical and microbiological analysis, waterborne pathogens.

## Introduction

Groundwater makes up about 20% of the world's supply. Wells are one of the major sources of ground water. In many places wells provide a reliable and ample supply of water for drinking, irrigation, and industries (Nair et al., 2013). During the last decade, it was observed that the ground water got polluted drastically because of increased human activities (Jameel, 1998). Sewage and wastewater could potentially contaminate wells and thus the drinking water. As per World Health Organisation (1993) standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Microbiological water analysis is mainly based on the concept of faecal indicator bacteria (Gopinath et al., 2012). The coli form test allows the use of coli forms as an indicator for routinely evaluating drinking water for the presence of pathogens. The coli form test first indicates either the presence or absence of total coli forms. The principle bacterial pathogens that have been shown to cause human intestinal disease associated with drinking water are *Salmonella typhi*, *Salmonella paratyphi-A*, other *Salmonella* species, *Shigella* sp., *S. flexneri*, and *S. sonnei*, *Vibrio cholerae*, *Leptospira* sp., *Yersinia enterocolitica*, *Francisella tularensis*, *Escherichia coli* (specific strains) and *Pseudomonas aeruginosa*, etc. (National Research Council 1987; Chandran, 2014). In order to eliminate the presence of such pathogens, chemical disinfectants are used. Chlorine is one such example and it is effective against bacteria and viruses. Because of the importance of good drinking water and an attempt was made to study the physicochemical and microbiological quality of well water samples from Punnapra village.

## Materials and Methods

### Sample Collection

Ten well water samples were collected from wells of North Punnapara, Alappuzha district, Kerala State. Ten samples were collected from Manthazha, Chennakalmadam, Brightland school, Ikkarachira, Pathinanchil, Vaadakkal, Bhagavathikkal, Panayakulangara, Block, Kalarcode. Samples were collected during the month of December from wells more than 1 kilometre radius apart. Water samples were collected in one litre sterilized bottles and it is transported in icebox to the laboratory within 4 hours and then processed.

## Organoleptic Analysis of Water Samples

The following organoleptic characters such as odour, colour and taste of each water samples are analysed (Dietrich and Burlingame, 2015).

### Physical Parameters

The pH of all water samples were checked using pH meter (Systronics 361, India). The temperature was measured using standard mercury filled centigrade thermometer. The electrical conductivity and Total Dissolved Solids (TDS) were measured using pre-calibrated conductivity TDS meter (Systronics 308, India). Salinity and specific gravity of the water samples were estimated using a handheld refractometer (Erma, ERS10, Tokyo Japan).

### Chemical Parameters

Alkalinity, hardness, BOD and COD were analysed using standard methods (APHA, 1992).

### Microbiological Analysis

#### Heterophilic Plate Count

Heterophilic plate count (HPC) can provide an indication of general microbial population in water. Samples to be analyzed for quantitative bacterial analysis, were plated on Glucose Tryptone (GT) Agar (APHA 1998), fungal analysis were done on Potato dextrose agar and the Actinomycetes count was estimated using Kenknight and Munaier's medium. The total plate count was performed after incubation at 37°C for 24 hours.

#### Most Probable Number (MPN)

The Most Probable Number multiple or multiple tube fermentation technique was used for coliform enumeration. In the presumptive test for coliforms, three 10 ml, three 1ml, and three 0.1 ml volumes of the appropriate dilution of water samples were inoculated in respective nine fermentation tubes and inverted Durham tubes were also placed in lactose broth to detect gas production. The inoculated test tubes were incubated for 48 h at 37°C, and those containing air bubbles were confirmed by plating on Eosin Methylene Blue agar (EMB) at 37°C for total coliforms (APHA 1992).

### Biochemical analysis

The organisms in water samples were Gram stained and further confirmed by IMViC (Indole, Methyl Red, Vogues Proskeur and Citrate utilization) tests (Cappucino and Sherman, 2002).

### Bacteriological Analysis of Water Sample

Bacteriological analysis was done to detect the presence of water borne pathogens such as *Salmonella*, *Vibrio*, *E.coli* etc. The samples were plated on specific agar medium such as Bismulth Sulfate Agar (BSA), Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS) and Mac Conkey agar.

### Results

#### Organoleptic Analysis of Water Sample

The collected water samples showed different colour variations such as colorless, yellow and brown (Table

1). The water samples collected from Manthazha, Pathinanchil, Vaadakkal, Bhagavathikkal, Panayakulangara and Block had an earthy odour and there was no odour for the water sample collected from Chennakkalmadam, and Ikkarachira. The water sample from Brightland School had a chlorous odour and the sample from Kalarcode had an earthy odour (Table 1).

The water sample collected from Manthazha, Pathinanchil, Bhagavathikkal, Panayakulangara and Block are of earthy taste. There was no taste for the water samples collected from Chennakkalmadam, and Ikkarachira. There was a chlorous taste for the water samples collected from Brightland School. The water sample collected from Vaadakkal has a muddy taste. The findings of other samples are given in table 1.

**Table1:** Organoleptic character analysis of water samples

Sample number	Place	Odour	Color	Taste
1	Manthazha	Earthy	Colorless	Earthy
2	Chennakkalmadam	Inodorous	Colorless	Tasteless
3	Brightland school	Chlorous	Colorless	Chlorine
4	Ikkarachira	Inodorous	Colorless	Tasteless
5	Pathinanchil	Earthy	Colorless	Earthy
6	Vaadakkal	Earthy	Brown	Muddy
7	Bhagavathikkal	Earthy	Colorless	Earthy
8	Panayakulangara	Earthy	Colorless	Earthy
9	Block	Earthy	Yellow	Earthy
10	Kalarcode	Earthy	Yellow	Dirty

#### Physical Parameter Analysis

The pH of the water samples ranged from 6 to 7.14 and temperature ranged from 27.4 to 27.5. The electrical conductivity ranged from 156  $\mu$ s to 625  $\mu$ s

and the water sample collected from Chennakkalmadam showed an EC value of 1.040 mS. EC values obtained for other samples are given in table 2. Salinity and specific gravity was zero for all the water samples collected.

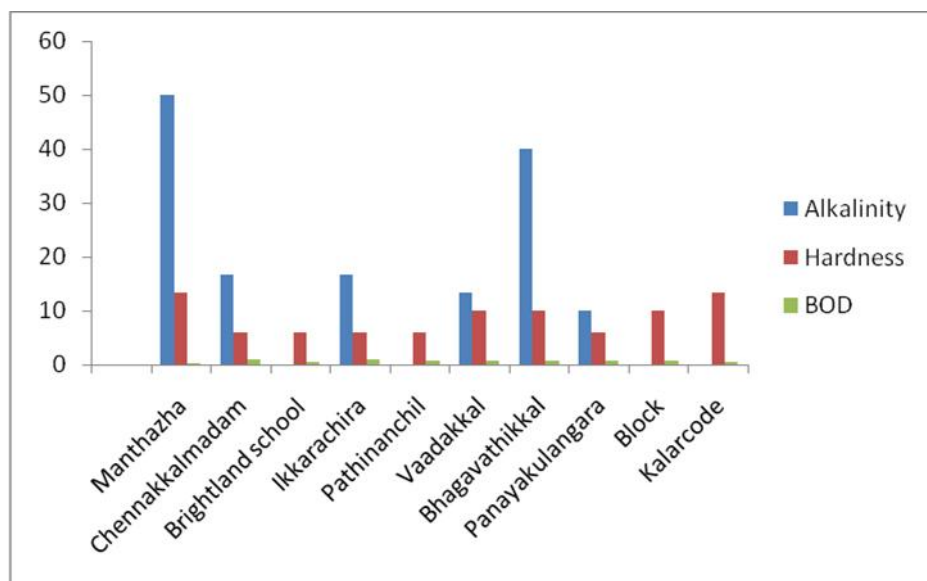
**Table 2:** Physical parameters of water samples

Sl. No.	Place	pH	Temperature (°C)	Electrical conductivity (µs/cm)	TDS (µs)	Salinity (psu)	Specific gravity
1	Manthazha	6.47	27.4	625	34.29	0	0
2	Chennakkalmadam	7.14	27.5	1.040 mS	58.64	0	0
3	Brightland school	6.21	27.5	156.0	94.78	0	0
4	Ikkarachira	6.74	27.5	368.8	208	0	0
5	Pathinanchil	6.00	27.5	383.0	214.2	0	0
6	Vaadakkal	6.51	27.5	307.4	169.5	0	0
7	Bhagavathikkal	6.67	27.5	238	130.2	0	0
8	Panayakulangara	6.87	27.5	239	131.5	0	0
9	Block	6.43	27.5	470	261.3	0	0
10	Kalarcode	6.56	27.5	528.3	297.5	0	0

**Alkalinity, Hardness, BOD of Water Samples**

Alkalinity was not found in water sample collected from 4 different locations such as Brightland School, Pathinanchil, Block, Kalarcode. Hardness of water

samples ranged from 6 ppm to 13.3 ppm. The lowest value for BOD 0.3 mg/L was recorded from Manthazha and the highest was 1mg/L recorded from Ikkarachira (Figure 1).



**Figure 1:** Alkalinity, Hardness and BOD of water samples

**Determination of COD**

The COD value ranged from 136 mg/L (Kalarcode) to 230.4 mg/L (Bhagavathikkal). COD for other places are given in the figure 2.

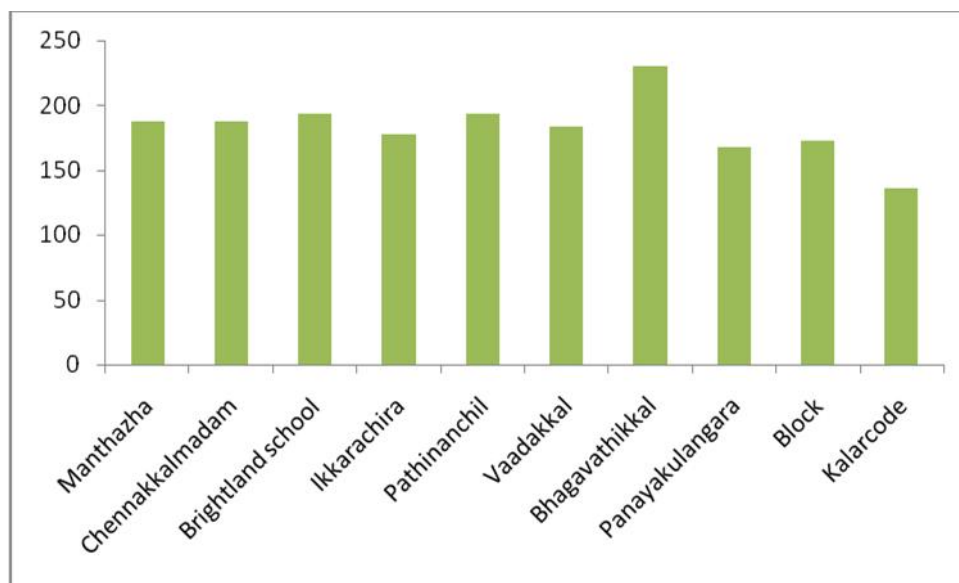


Figure 2: COD of water samples

### Heterophilic Plate Count

The lowest heterophilic bacterial count was  $2 \times 10^3$  cfu/ml was recorded from Brightland School and the highest was  $43 \times 10^3$  cfu/ml recorded from Ikkarachira, while the lowest fungal count,  $4.66 \times 10^2$  cfu/ml was recorded from Panayakulangara and the

highest fungal count,  $49 \times 10^2$  cfu/ml was recorded from Bhagavathikkal. Lowest actinomycetes count ranged from  $2 \times 10^3$  cfu/ml to the highest  $21.66 \times 10^3$  cfu/ml and there occurred the absence of actinomycetes in water samples collected from Brightland School and Panayakulangara.

Table 3: Heterophilic plate count of microorganisms

Sample number	Place	Heterophilic Plate Count (HPC)		
		Bacteria (cfu/ml)	Fungi (cfu/ml)	Actinomycetes (cfu/ml)
1	Manthazha	$4.33 \times 10^3$	$10.33 \times 10^2$	$21.66 \times 10^3$
2	Chennakkalmadam	$26 \times 10^3$	$32.66 \times 10^2$	$9 \times 10^3$
3	Brightland school	$2 \times 10^3$	$23 \times 10^2$	0
4	Ikkarachira	$43 \times 10^3$	$23.33 \times 10^2$	$4.33 \times 10^3$
5	Pathinanchil	$37.33 \times 10^3$	$16 \times 10^2$	$7.33 \times 10^3$
6	Vaadakkal	$8 \times 10^3$	$8.33 \times 10^2$	$11 \times 10^3$
7	Bhagavathikkal	$17.33 \times 10^3$	$19 \times 10^2$	$7 \times 10^3$
8	Panayakulangara	$4 \times 10^3$	$4.66 \times 10^2$	0
9	Block	$17.33 \times 10^3$	$7 \times 10^2$	$2 \times 10^3$
10	Kalarcode	$15 \times 10^3$	$11.33 \times 10^2$	$3 \times 10^3$

### Most Probable Number

Fermentation tubes were examined for the production of acid and gas production after 24-48 hours of incubation. The test samples which showed positive

results for presumptive test were further confirmed by confirmed and completed tests. The lowest MPN index observed was 23 from Bhagavathikkal and the highest was 2400 from Kalarcode.

**Table 4:** Most probable number of water samples

Sample number	Place	MPN index per 100 ml
1	Manthazha	460
2	Chennakkal madam	240
3	Brightland school	93
4	Ikkarachira	240
5	Pathinanchil	240
6	Vaadakkal	93
7	Bhagavathikkal	23
8	Panayakulangara	1100
9	Block	240
10	Kalarcod	2400

### Gram Staining and IMViC

Gram staining and IMViC tests confirmed the presence of *E. coli* in samples from Manthazha, Chennakkal madam, Ikkarachira, Pathinanchil, Vaadakkal and Kalarcod (Table 5).

**Table 5:** Results of Gram staining and IMViC Test

Sample number	Place	Gram reaction	Indole test	Methyl red test	Voges-proskauer test	Citrate test
1	Manthazha	-	+	+	-	-
2	Chennakkal madam	-	+	+	-	-
3	Brightland School	+	-	-	+	+
4	Ikkarachira	-	+	+	-	-
5	Pathinanchil	-	+	+	-	-
6	Vaadakkal	-	+	+	-	-
7	Bhagavathikkal	+	-	-	+	+
8	Panayakulangara	+	-	-	+	+
9	Block	+	-	-	+	+
10	Kalarcod	-	+	+	-	-

### Isolation and Identification of Pathogenic Bacteria

The presence of *E. coli* and other enterobacteriaceae members in water samples were identified on the basis

of colony morphology on EMB agar. *E. aerogenes* and *E. coli* was found only in six places i.e. Manthazha, Chennakkal madam, Ikkarachira, Pathinanchil, Vaadakkal and Kalarcod (Table 6).

**Table 6:** Presence or absence of *E. coli* in water samples plated on EMB Agar

Sample number	Place	Eosin methylene blue agar (“+” indicate presence and “-” indicate absence)	
		<i>E. aerogenes</i>	<i>E. coli</i>
1	Manthazha	+	+
2	Chennakkalmadam	+	+
3	Brightland School	+	-
4	Ikkarachira	+	+
5	Pathinanchil	+	+
6	Vaadakkal	+	+
7	Bhagavathikkal	+	-
8	Panayakulangara	+	-
9	Block	+	-
10	Kalarcode	+	+

The presence of *E. coli*, *E. aerogenes* and *Enterococcus faecalis* in water samples were confirmed in Mac Conkey agar. *E. aerogenes* were found in samples collected from Manthazha,

Chennakkalmadam, Bhagavathikkal, Panayakulangara. *E. faecalis* were present in water samples from Block and Kalarocde (Table 7).

**Table 7:** Presence or absence of pathogens in water samples plated on Mac Conkey Agar

Sample number	Place	Mac Conkey agar (“+” indicate presence and “-” indicate absence)		
		<i>E. coli</i>	<i>E. aerogenes</i>	<i>E. faecalis</i>
1	Manthazha	+	+	-
2	Chennakkalmadam	+	+	-
3	Brightland School	-	-	-
4	Ikkarachira	+	-	-
5	Pathinanchil	+	-	-
6	Vaadakkal	+	-	-
7	Bhagavathikkal	-	+	-
8	Panayakulangara	-	+	-
9	Block	-	-	+
10	Kalarcode	+	-	+

TCBS agar is used for the isolation of *Vibrio cholerae* and other enteropathogenic *Vibrios*. The water samples collected from 3 different locations such as Manthazha, Panayakulangara and Block showed the presence of *Vibrio cholerae*. The water sample collected from 6 different locations such as Manthazha, Chennakkalmadam, Brightland School,

Ikkarachira, Panayakulangara and Kalarcode showed the presence of *Vibrio parahaemolyticus*. The water sample collected from 7 different locations such as Manthazha, Brightland School, Pathinanchil, Vaadakkal, Bhagavathikkal, Panayakulangara and Kalarcode showed the presence of *Vibrio vulnificus* (Table 8).

**Table 8:** Presence of pathogens in TCBS agar

Sample number	Place	Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar		
		<i>Vibrio cholerae</i>	<i>Vibrio parahaemolyticus</i>	<i>Vibrio vulnificus</i>
1	Manthazha	+	+	+
2	Chennakkal madam	-	+	-
3	Brightland School	-	+	+
4	Ikkarachira	-	+	-
5	Pathinanchil	-	-	+
6	Vaadakkal	-	-	+
7	Bhagavathikkal	-	-	+
8	Panayakulangara	+	+	+
9	Block	+	-	-
10	Kalarcod	-	+	+

BS agar is used as the specific medium for the isolation of *Salmonella typhi*. The *S. typhi* was found in 7 different locations such as Manthazha,

Chennakkal madam, Brightland School, Ikkarachira, Pathinanchil, Panayakulangara and Block (Table 9).

**Table 9:** Presence or absence of pathogens in BSA

Sample number	Place	Bismuth Sulphite Agar (BSA)
		<i>S. typhi</i>
1	Manthazha	+
2	Chennakkal madam	+
3	Brightland School	+
4	Ikkarachira	+
5	Pathinanchil	+
6	Vaadakkal	-
7	Bhagavathikkal	-
8	Panayakulangara	+
9	Block	+
10	Kalarcod	-



## Discussion

Water the most precious resource of the earth. The current study focused on the physicochemical and microbiological analysis of well water samples collected from North of Punnapra village, Alappuzha district, Kerala state, India. The organoleptic characteristics of water samples were analysed to recognise the acceptability of each water samples. pH, temperature, electrical conductivity, total dissolved solids, salinity and specific gravity of 10 different water samples were also analysed and the results were similar to the work done by (Athira et al., 2019). The chemical parameters such as alkalinity, hardness, BOD and COD were compared on the basis of values recognised by (BIS). 1991. The HPC of all water samples revealed that sample collected from Ikkarachira had more number of bacterial colonies on culture media and less number of bacterial colonies were found in the sample collected from Brightland school, which is similar to the work done by (Chandran et al., 2011). The CFU value of HPC of all water samples revealed that sample collected from Chennakkalmadam had the highest value of fungal colonies and the lowest value of fungal colonies were found in the sample collected from Panayakulangara (Pavlov et al., 2004). The CFU value of HPC of all water samples revealed that sample collected from Manthazha had the highest value of actinomycetes colonies. HPC can provide an indication of the level of the general population in the system and is considered as a good general indicator of overall water quality (Obire et al., 2005). MPN is most commonly applied for quality testing of water. A group of bacteria commonly referred as fecal coliform act as indicator for fecal contamination of water (Humbert et al., 1997). The isolation and identification of pathogens were done on the basis of biochemical test and morphological analysis on the specific agar surface which is similar to the work done by (Thamidela et al., 2017). The presence of *E. coli*, *S.typhi* and *V. cholerae* pose a threat to the residents in the area.

## Conclusion

In the present study it is concluded that the water samples are contaminated with pathogenic microorganisms and the water samples are unfit for drinking unless it is boiled.

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## References

- American Public Health Association (APHA). 1998. Standards Methods for the Examination of Water and Wastewater. 20<sup>th</sup> Edn, American Public Health Association, Washington, D.C.
- American Public Health Association (APHA). 1992. Standard Methods for the Examination of Water and Waste Water. 18<sup>th</sup> Edn. Washington, D.C.
- Athira, T.S., Vijayan, B., Gopika, G., George, T., Sudhakaran, R., Franklin, N. and Chandran, P. R. 2019. Screening and Identification of Pathogenic Microorganisms in Backwaters of Alappuzha District, Kerala State, India. Int. J. Adv. Res. Biol. Sci. 6(4): 62-69.
- Bureau of Indian standard. (BIS). 1991. Guidelines for Indian drinking water Quality.
- Cappucino, J.G and N. Sherman. 2002. Microbiology: Laboratory Manual . Benjamin Cumming Publishing Co. California USA. pp. 152-187.
- Chandran, P. R., Kiran K., Divakaran, D. and Prajisha, P. K. 2011. Analysis of bacteriological quality of drinking water samples from Cherthala taluk, Kerala, India. Asian J. Water. Environ. Pollut. 8 (4): 61-68.
- Chandran, PR. (2014). Harboring of pathogenic microorganisms by aquatic weed, *Eichhornia crassipes* in its rhizosphere. Int. J. ChemTech Res 6 (2), 1413-1417.
- Dietrich, A. M. and Burlingame, G. A. 2015. Critical Review and Rethinking of USEPA Secondary Standards for Maintaining Organoleptic Quality of Drinking Water. Environ Sci Technol. 49(2), 708-720.
- Gopinath, A., Chandran, P. R., Vysakhi M.V. and Anu S. (2012). Physical and bacteriological quality of well water samples from Kanakkary Panchayath, Kottayam District, Kerala State, India. Int. J. Pl.An and Env.Sci. 2 (3): 133- 138.
- Humbert, F.S., Salvat, G., Lalande, F., Colin, P., 1997. Miniaturezed most probable number and enrichment serology techniques for the enumeration of Salmonella spp. pp. 1306-1311.

- Jameel, A., 1998. Physico chemical studies in Vyyakondan Channel water of Cauvery. Poll. Res., 17Q 111-114.
- Nair, G.A., Chandran, P. R., Sukumar, B., Santhosh, S., Vijayamohanan and Sobha, V. 2013. Assessment of well water quality in Tsunami affected regions of south-west coast of Kerala, India. J. Environ. Biol. 34 (4): 771 - 777.
- National Research Council: Drinking Water and Health Disinfectants and Disinfectant By-Products. National Academy of Sciences, Volume 7, National Academy Press, 1987.
- Obire, O., Tamuno, D.C. and S.A. Wemedo., 2005. Bacteriological Water Quality of Elechi Creek in Port Harcourt, Nigeria. J. Appl. Sci. Environ. Mgt., 9(1):79-84.
- Pavlov, D., de wet, C.M., Grabo, W.O., Ehlers. M.M. 2004. Potentially pathogenic features of heterophilic plate count bacteria isolated from treated and untreated drinking water. Int. J. Food microbial. 92 (3):275-87.
- Sirisha, T., Basha, P.A., Kavitha, B. 2017. Isolation and characterization of pathogenic Bacteria from Kundu River water of Nandyal, Kurnool, Andhra Pradesh, India. J. Appl. Sci. 17(9): 475-481.
- WHO 1993. Guidelines for Drinking Water Quality, Volume I, II and III. World Health Organization, Geneva.

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