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Detection of Coliform, faecal Coliform And Total Bacterial Count From Drinking Water Of Varanasi (U.P.) India

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ABSTRACT

In India source of drinking water at Varanasi city for common people are tap water, well, hand pump, Ganga river and stored tank water collected from bore well. All water samples were studied to assess their bacteriological characteristics and suitability for potable purposes. A cross-sectional epidemiological method was adopted to investigate the drinking water of six different sites of Varanasi city. The bacteriological examination of water samples included the most probable number of presumptive coliforms, faecal coliforms, and total bacterial count. The results showed that the total coliform count was detected in all the site. In all the methods coliforms presence was indicated. Maximum number of coliform observed in all the seasons, were from river and well water followed by hand pump, tap water and stored tank. The most common group of indicator organisms used in water quality monitoring are coliforms. These organisms are representative of bacteria normally present in the intestinal tract of mammals including human. Contamination of water may occur through different way like sewage disposal in the river, seepage of bathing near sites, fecal excreta of human, bird and other animals. Improving and expanding the existing water treatment and sanitation systems are more likely to provide good, safe and sustainable sources of water in the long term.

Figures : 03 References : 29 Table : 00
KEY WORDS : Coliform, Disease, Indicator, Water.

Introduction

In India 12% of people get clean drinking water, the rest 88% quench their thirst from polluted lakes, tanks, rivers and wells due to which more than three million people get affected or die of enteric diseases every year. Those who have privilege of having river in their villages, it becomes the source of drinking water. This river water is polluted by the animals that slake their thirst, by dhobis who wash the clothes, by the garbage of the city and others. Even piped water which is available in big cities, is also mixed with number of impurities causing jaundice, cholera, typhoid and gastroenteritis. Rivers are the major source of drinking water for people in the rural areas. These rivers are getting increasingly polluted by city waters. Today, infectious diseases cause approximately 37% of all deaths worldwide[20]. In addition, more than 4 million infants and children die each year from diarrhea, which is caused largely by contaminated water and food [25,26]. Water borne infections account for 80% of all infectious diseases world wide and 90% of all infectious diseases in developing countries[8]. Lack of sanitary conditions contributes to approximately 2 billion human infections of diarrhoea, resulting in approximately 4 million deaths each year, mostly among infants and children [25]. Even in developed countries, waterborne diseases are significant. In the United States, they account for 940,000 infections and approximately 900 deaths each year.

Furthermore, 114 cities dump untreated sewage and partially cremated bodies directly into the sacred Ganges River. Then, downstream, the untreated water is used for drinking, bathing and washing. This situation is typical of many rivers in developing countries. Workers[19] isolated *Salmonella typhi* from tube well water in and around Hyderabad. The most concentrated opportunistic pathogens in urban storm water around the Baltimore area[16] were found to be *Pseudomonas aereginosa* and *Staphylococcus acercus* respectively. Investigators[17] recorded standard plate count, total coliform and faecal

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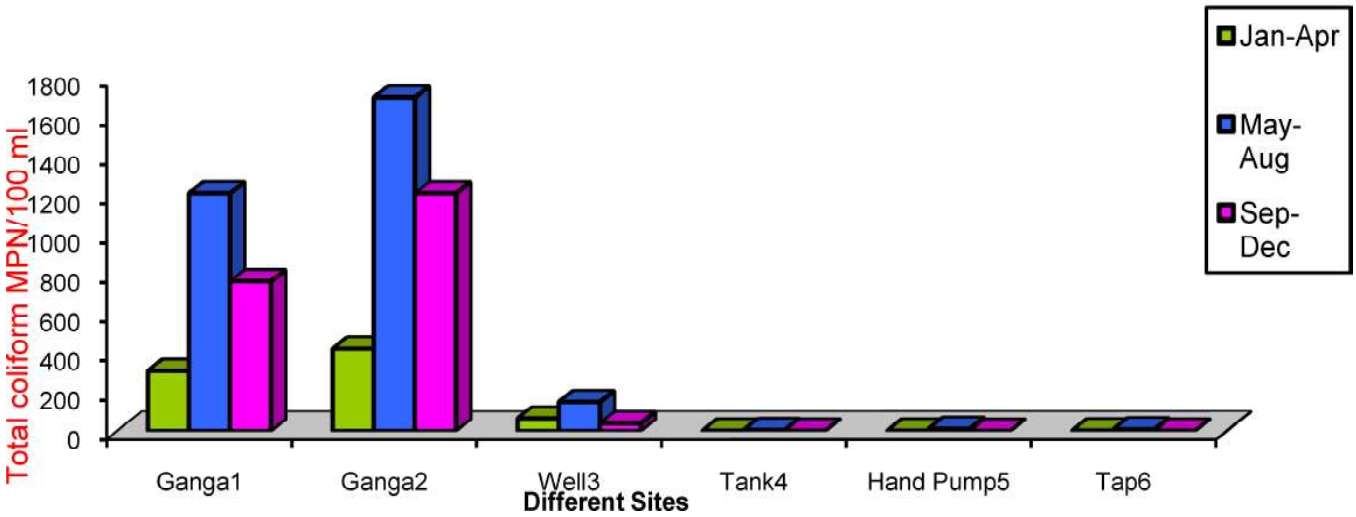

Fig. 1 : Seasonal Variation in total coliform count

coliform in water of Oaty lake, Udagymandalam. Others[21] investigated 150 drinking water samples from 35 different villages of Gujarat and reported that except three tube well water samples 74.2% of the dug well water samples, 66.6% of house water samples and 50% of the pond water samples showed the presence of enteropathogens. A variety of waterborne disease outbreaks have been attributed to untreated or poorly treated ground water containing pathogenic bacteria, viruses or eukaryotic organisms. Another study from urban areas of Dhaka city shows that the faecal coliform count/100 ml of water (bacteriological count) of piped water sample is significantly higher than that of tube well water samples. Diarrhoea due to *Vivrio cholerae* non-01 is also common in Bangladesh and other parts of the world[11,15]. Epidemic due to *V. cholerae* non-01 have also been reported during choleral outbreaks[7]. Drinking water is one of the possible sources of *Acromonas* related diarrhoea[10]. In the Netherlands *Aeromonas* spp. are frequently present in drinking water in numbers varying between <1 and 10,000 cfu/100 ml[9] and this organism may be isolated from 1.6% of faecal sample of patients with diarrhoea with a distinct summer peak up to 3%. Some bacterial species may be indicator of pollution, for instance the presence of coliform organism in water is an indicative of the water being contaminated with faecal matter[14]. In India, large population particularly in rural areas suffers from water borne and water related diseases and need constant monitoring. In a WHO reports it has been reported that 80% of all sickness and diseases in third world are due to consumption of contaminated water. It was detected that there were pathogenic bacteria in natural resources as well as piped water of Municipal Corporation, Shimla which are used for drinking purposes[19].

The most important source of drinking water for about 70 percent of Indian population is ground water and is considered less polluted. But lack of sanitation, improper waste disposal, faulty well construction and lack of water source protection increase ground water contamination and 40 percent or more of the disease outbreaks were attributed to polluted groundwater consumption. The enumeration of viable count, aerobic heterotrophs, coliforms and *Escherichia coli* generally indicate the extent of water pollution and have attracted the waters to assess the microbial quality of water. Others[18] estimated 15 well water samples from rural area of Bareilly and Nainital districts for Total Heterotrophic Bacteria (THB), Total Coliforms (TC), Faecal Coliforms (FC) and *E. coli* type I (ECI). These enteric bacteria are indicative of recent fecal contamination and the possible presence of pathogenic microorganisms[5] although there is no simple correlation between concentration of indicator bacteria, the presence of microbial pathogens, and risk of illness. A number of workers[27-29] have analysed and discussed the bacteriological characteristics of river water at various places. Many workers have reported MPN coliform and *E. coli* of human origin from Ganga, Yamuna and Sangam at Allahabad as high as $65x10^3$, $55x10^3$ and $15x10^2$, $12x10^2$, $11.5x10^2$/100 ml, respectively. For microbial examination of drinking water (IS:1622, 1969) the specification includes 1 coliform/100 ml as the limit for water to be used in industry. The new regulation requires that these should be 0 coliform per 100 ml by any method for any sampling frequency for drinking water. World Health Organization allows to 10 coliform per 100 ml for small community water sources. The prevention of drinking water free from microorganisms is one of the first tasks to be undertaken in the introduction of environmental sanitation.

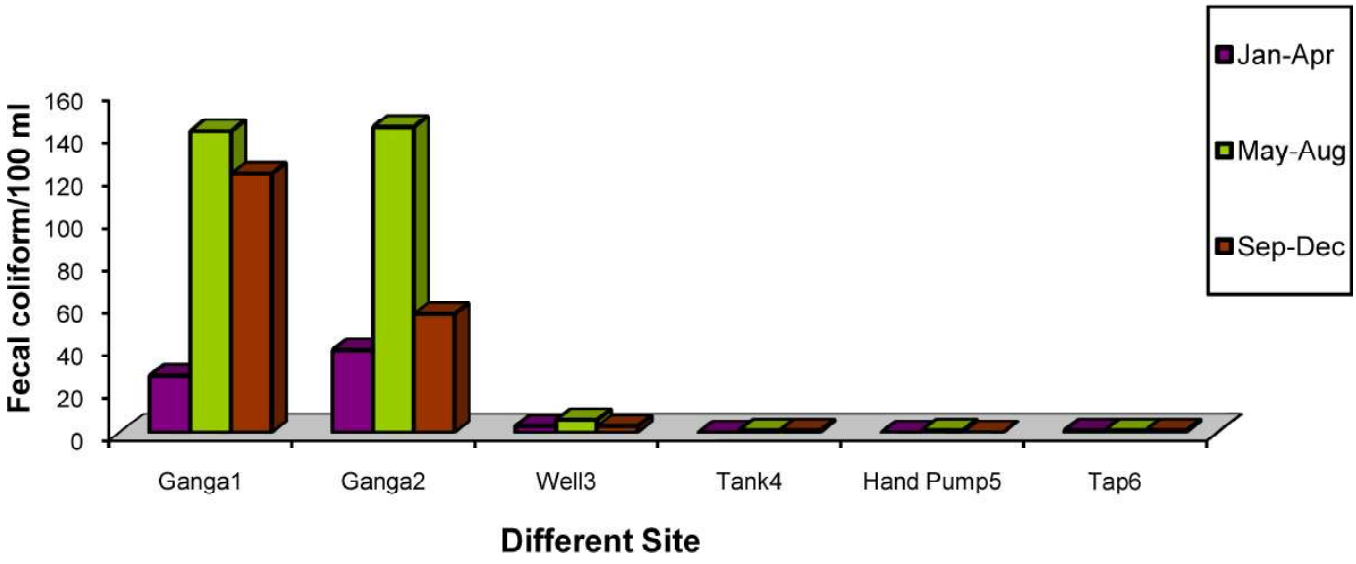

Fig. 2 : Seasonal Variation in faecal coliform count

Materials and Methods

Description of the sites and sample collection

Samples of water were collected from six different sites of Varanasi city. All Samples were collected in sterile sample collection bottles and labeled with respective sample number. Two sites were made along the bank of the river. Site first *viz.* University Ghat situated near BHU, and negligibly polluted. Hundreds of people of Varanasi as well as pilgrims take bath here and wash their cloths daily. Site second *viz.* Assi Ghat at about 1 km downstream from the site first. Here a continuous discharge of city sewage through a large drain occurs on the bank of the river. Site third is a cemented tank situated in a house of village Karaungi which is one km away from BHU. In this tank, water is filled by borewell. This tank has a lid at the tap, which open outside. Site fourth is a well situated in the same village around which a large number of people take bath and wash their cloths daily. They also use this water for drinking. The well is about 40 feet in depth. Site fifth is a hand pump situated in village Sunderpur with a depth of about 35 feet. Site sixth is a tap situated near the municipal water supply strata of Varanasi city. This tap alone have water supply of municipal corporation. The mouth of the bottle and the stopper were flamed with spirit lamp before and after collection of water sample to rule out the possibility of contamination while handling.

Bacteriological Examination of Water

Six sampling stations were fixed for regular collection and bacteriological examination of water samples at the rate of once every month for the year 2018-2019. The routine tests used in bacteriological examination of water are:

(1) Total Coliform Count :

This estimation was made by adding measured amounts of single and double strength modified MacConkey fluid medium in sterilized tubes containing a Durham tube for indicating gas production. The size of the tube varies with the quality of medium and water to be added to it. With sterile pipettes the following amounts of water were added:

One 50 ml quantity of water to 50 ml double strength medium.

Five 10 ml quantities each to 10 ml double strength medium.

Five 1 ml quantities each to 5 ml single strength medium.

This range of quantities could be altered or changed according to the likely condition of water examined. The tubes were incubated at 37^0C and examined after 18-24 h. Those that showed acid and sufficient gas to fill the concavity at the top of the Durham tube were considered to be presumptive positive as a result of growth of coliform bacilli. Any remaining negative tubes were reincubated for another 24 hrs and if acid and gas develop they too were regarded as being positive. In reporting the results of the presumptive test reference was made to McCrady's probability Tables.

(2) Faecal Coliform Count:

Some spore bearing bacteria gave false positive reaction in the presumptive coliform test (MPN count)or total coliform count. The Eijkman test was used for this purpose; as it gives valid results with inocula of mixed bacteria from the cultures grown in the presumptive coliform test (MPN count) and does not

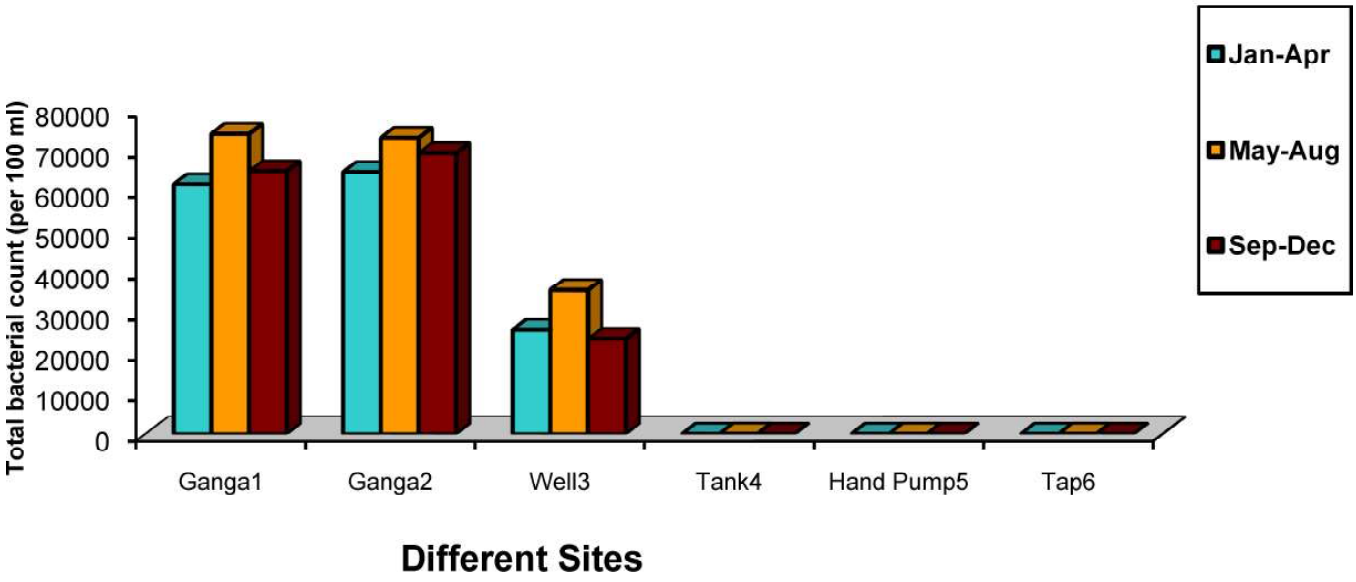

Fig. 3 : Seasonal Variation in Total Bacterial count

require the preliminary isolation of the bacteria in pure culture. The test was done by incubating the subcultures from the positive presumptive test at 44^0C and 37^0C for 24 hrs in a lactose containing medium inhibitory to spore forming bacteria *e.g.* lauryl tryptone broth or brilliant green lactose bile broth and other sub cultures at 44^0C in tryptone water. The presence of coliform bacilli was confirmed by the production of gas from lactose at 37^0C. That of *E. coli* was confirmed by the production at 44^0C of gas from lactose and indole from tryptophan. From the combination of positive and negative results for gas production, read off (from MPN Tables) the coliform bacilli per 100 ml of water.

(3) Total Bacterial Count (Colony count)

A total bacterial count was made by calculating the number of colonies appearing per nutrient agar plates incubated at 37^0C for 24 hours to which aliquot of water samples were added. Serial dilutions were made from the water samples *i.e.* 1:0, 1:10, 1:100. Nutrient agar medium in the petridish mixed well with the desired dilutions of the samples and were incubated at 37^0C for 24 h. Each volume or dilution were plated in duplicate. After incubation the colonies that have developed in the medium were counted in a colony counter.

$$\text{SPC/ml} = \frac{\text{Colonies counted}}{\text{Dilution factor}}$$

Result and Discussion

There were considerably high values from May to Aug at site II in the study period. In all six sites, maximum value of total coliform was recorded $17x10^2$/100ml (May-Aug) at site II. The minimum value recorded was nil at site V. But the site wise variations were 1212/100ml (May-Aug) to 306.25/100ml (Jan-Apr) at site I, $17x10^2$/100ml (May-Aug) to 418.75/100ml (Jan-Apr) at site II, 147.5/100ml (May-Aug) to 38.75/100ml (Sep-Dec) at site III, 4/100ml (May-Aug) to 0.75/100ml at site IV,7/100ml (May-Aug) to nil (Jan-Apr and Sep-Dec) at site V, and 7.5/100ml (May-Aug) to 1/100ml (Sep-Dec) at site VI (Fig.1).

High densities of Faecal Coliform were recorded at site I as compared with other sites. There were considerably high values from May-Aug and Sep-Dec in the study period. In all the six sites, maximum mean value of faecal coliform was observed 143.2/100ml at site II in May-Aug. The minimum values of faecal coliform recorded were nil at site IV and V in Jan-Apr. But at site variations were 142/100ml (May-Aug) to 52.75/100ml (Sep-Dec) at site I, 143.2/100ml (May-Aug) to 38.75/100ml at site II, 6/100ml (May-Aug) to 2.25/100ml at site III , 0.5/100ml (May-Aug) to nil (Jan-Apr) at site IV, 0.75/100ml (May-Aug) to nil (Jan-Apr) at site V and 0.5/100ml (May-Aug) to 0.25/100ml (Jan-Apr) at site VI (Fig. 2).

High densities of total bacterial count were recorded at site I as compared to other sites. There were considerably high values from May-Aug of site I and II in the study period. In all six sites, maximum value of total bacterial count was recorded 74000 per 100ml at site I from May-Aug. the minimum value recorded was nil at site V from Jan-Apr and from Sep-Dec. But the site wise variations were 74000/100 ml (May-Aug) to 64750/100ml (Sup-Dec.) at site I, 73x103/100 ml (May-Aug) to 2950/

100 ml (Sep-Dec.) at site II, 355x103/100 ml (May-Aug) to 23.5x103/100 ml (Sep.-Dec) at site III, 41.2/100 ml (May to Aug) to nil (Jan- Apr) at site IV, 23/100 ml (May - Aug) to nil (Sep-Dec) at site V and 18.5/100 ml (May – Aug) to 2.5/100 ml at site VI (Fig. 3).

Bacteriological quality of water to a very great extent depends upon the qualitative and quantitative pattern of bacteria. The concentration of these organisms varied with the organic load, season, water current, depth, nature of pollutant and physicochemical quality of water[18].A large number of township and industries are located in the middle and lower strength of the Ganga river (*i.e.* near site I and II). Untreated waste from the industries and municipalities were discharged directly in the river which result in high concentration of bacteria. Total bacterial count, MPN of total coliform and faecal coliform were recorded from all the six sites. Many workers[23,24] considered coliform as a reliable indicator of contamination of pollution.

In the present study total count, MPN of total coliform and faecal coliform were low during Jan-Apr and high during May–Aug and Sep-Dec. During May-Aug abundance was attributed to higher concentration of organic matter, low water level and high temperature. Further few sites (I, II) of river Ganaga have bathing Ghats where disturbances due to high human activities *i.e.* continuously discharge of human sewage, accumulation of night soil and bathing and washings were also seen during summer months. Such types of activities were also seen at well (site III). Similar observations were made by different workers[18] and stated that bacteria will always and at all conditions increase in number after heavy rain who further supported that the total number of the living organisms would give some indication as to the amount of organic matter present in the water and their nutritional requirement. Nil value of total bacteria count was recorded from site IV (during Jan-Apr) and from site V (during Sep-Dec) whereas nil value of MPN of total coliform was recorded at site V (during Sep-Dec). Similar observation was recorded from faecal coliform except that nil value was also observed during Jan-Apr from site V. If we compare the bacteria load between site IV (Storage tank), V (Hand pump) and VI (Municipal water supply), minimum load was reported from site VI, followed by IV and V, but it is within the permissible limit as per recommendations of ICMR and World Health Organization. The MPN/100 ml in water should not exceed 10 (*E. coli* should be nil). Similar type of observation was recorded by different workers[3,4,22]. Presence of coliform from the site indicates that the water was not chlorinated properly. Graph indicate that bacterial presence and absence were variable throughout the study period at all the sampling sites. The possible reason for microbial contamination of the ground water may be excessive extraction of ground water which create vaccum thereby increasing the chances of suction of contaminated water. Besides this, the contaminated water may also mix with the potable water in the well through bathing and other human activities near well, seepage from manholes and soak pits adjacent to the ground water[4]. This may be one of the reasons for large number of gastroentrites cases in the states[3].

Conclusion

The link between the faecal contamination of water supplies and disease outbreak is now long established and sanitary indicator bacterial status is widely recognized as a fundamental measure of water quality by academic researchers[5] and the water industry[28]. These enteric bacteria are indicative of recent faecal contamination and the possible presence of pathogenic microorganisms[5], although there is no simple correlation between the concentration of indicator bacteria, the presence of microbial pathogens and the risk of illness. The total bacterial counts, total coliform count and faecal coliform count for all the water samples of all the sites were generally high exceeding the limit recommended by both Environmental Protection Agency (EPA) and World Health Organization (WHO) (of 1.0X102cfu/ml), which is the standard limit of heterotrophic count for drinking water. Therefore, appropriate treatment processes should, therefore be utilized for safe drinking water.

References

1. Baudart J, Grabulos J, Barusseau P, Lebaron P. *Salmonella* spp. and faecal coliform loads in coastal waters from a point *vs.* non-point source of pollution. *J. Environ. Qual.* 2000; **29**: 244-250.
2. Bukar AM, Isa MA, Mustapha A, Kyari MZ, Ibrahim FK. Bacteriological Analysis of Sachet Water in Maiduguri Metropolis. *The Journal of Applied Sciences Research;* 2015; **2**(1): 20-25.
3. Chakraborty BC. Bacteriological quality of water in some districts of Assam. *IJEP*. 1999; **19**(8): 587-589.
4. Cruz B, Priya GK, Nagarajan P. Water quality deterioration in Trichirappalli city. *IJEP.* 2000; Ground **20**(3): 171-173.
5. Craun GF. Disease outbreaks caused by drinking water. *J. Water Pollut. Control Fed.* 1975; **47**: 1566-1581.
6. Craun GF, Berger PS, Calderon RL. Coliform bacteria and waterborne disease outbreaks. *J. Am. Water Works*

Assoc. 1997; **89**(3): 96-104.

7. Dutt AK, Alwi S, Velanthan T. A shellfish-borne cholera outbreak in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1971; **65**: 815-818.
8. Epstein P, Ford T, Puccia C, Possas CDA. Marine ecosystem health. Implications for public health. Diseases in evolution, global changes and emergence of infectious diseases. *Annals of the New York Academy of Sciences.* 1994;**740**: 13-23.
9. Havelaar AH, Versteegh JFM, During M. On behalf of a working group The presence of *Aeromonas* in drinking water supplies in the Netherlands. *Zentralblatt für Hygiene*, 1990; **190**: 236-256.
10. Holmiberg SD, Schell WL, Fanning GR, Wachsmuth IK, Hickman-Brenner FW, Blake PA, Brenner DJ, Farmer III JJ. (1986). *Aeromonas* intestinal infections in the United States. *Annals of Internal Medicine*, 1990a; **105**: 683-689.
11. Hughes JM, Hollis DG, Gangarosa EJ, Weaver RE. Non-cholera vibrio infections in the United States, clinical, epidemiologic and laboratory features. *Annals of Internal Medicine*, 1978; **88**: 602-606.
12. Islam MS, Alam MJ, Neogi PKB. Seasonality and toxigenicity of *Vibrio cholerae* non – 01 isolated from different components of pond ecosystems of Dhaka city, Bangladesh. *World J. of Microbiol. and Biotech.* (1992); **8** : 160-163.
13. Mahajan KK. Deteriorating nation's rivers. In ecology and pollution of Indian rivers. Ed. R.K. Trivedi. *Asian Publishing House*, New Delhi. 1988; Pp. 1-38.
14. Mahanta KC. (Microbiology of water supply in the dairy plant. In dairy microbiology. 1984;. *Osmos Publications*, Rajourl Garden, New Delhi.
15. McIntyre OR, Feeley JE, Greenough III WB, Bnenenson AS, Hasan SI, Saad A. Diarrhoea caused by non-cholera vibrios. *American Journal of Tropical Medicine*.1995; **14**: 412-418.
16. Olivieri VP, Kruse CW, Kawata K. Microorganisms in urban stormwater. Environmental Protection Technology Series, *EPA*. 1977; 600/2-77-087, MERL, US Enironmental Protection Agency, Ohio.
17. Ramasubramanium R, Hariprasad V, Mohan R, Rao VNR. Microbial water quality of Oaty lake, Udagamandalam-A case study. *Pollution Research*. 1992; **11**(3): 117-121.
18. Rai H. The bacteriological studies on the Yamuna River (Delhi, India) *Sci. and papers from Inst. Chem. Technol. Prague*., 1964; **8** (1) : 319-336.
19. Rao PLKM, Reddy LVR. Distribution of heterotrophic and coliform bacterial flora in shallow ground water in Hyderabad metropolitan city, Andhra Pradesh. *Pollution Research*. 1995; **14**(2): 165-170.
20. Real LA. Sustainability and the ecology of infectious diseases. *Bio Science.* 1996; **46**: 88-97.
21. Shah PR, Patel HH. Diarrhoea causing enterepathogens in drinking water of rural Panchmahals of Guzarat, *Indian Environ. Hlth.* 1989; **31**(2): 171-172.
22. Thakur K, Grover PS, Gupta ML. Detection of pathogenic bacteria in drinking water by membrane filtration technique. *Indian J. Comm. Hlth.* 2000; **6**(1 and 2), 22-29.
23. Trivedy RK. Ecology and pollution of Indian Rivers, *Ashish Publishing House*, New Delhi, 1988; 1- 443.
24. Valecha V, Bhatnagar GP. Seasonal changes of phytoplankton in relation to some physico-chemical factors in Lower lake of Bhopal, *Geobios.* 1988; **15** : 170-173.
25. WHO. Standards for drinking water. World Health organisation. 1992; Geneva, Switzerland.
26. WHO. Bridging the Gaps. Geneva. 1995; World Health Organization.
27. Yadav NS, Kumar A, Sharma M. Ecological health assessment of Chambal river using water quality parameters. *J. Int. Sci. Technol.* 2014; **2** (23) : 52–56.
28. Yorkshir Water. Drinking water quality. 1993; Yorkshire Water, Leeds, U.K.
29. Zafari Y, Zarifi AZ, Rahmanzadeh S, Fakhar N. Diarrhoea caused by non-agglutinable *Virio choleras* (non-cholera vibrio). *Lancet.* 1973; **2**: 429-430.