EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/EA
EnvironmentAsia 6(1) (2013) 29-37

Occurrence and Distribution of Microbiological and Physico-Chemical Indicators in Ground Water Contaminated by Drainages, North India

Shreya Mishra [a], Sandeep Bisht [d], Roopa Malik [a], Jagvijay Singh [a], Priyanku Teotia [e], Shivesh Sharma [f], Ritu Kela [c], Vivek Kumar [b]

[a] *Department of Biotechnology, College of Applied Education & Health Sciences, Meerut-250003, India.*
[b] *Microbiology, College of Applied Education & Health Sciences, Meerut-250003, India.*
[c] *Biochemistry, Division of Biosciences, College of Applied Education & Health Sciences, Meerut-250003, India.*
[d] *Department of Applied Sciences, IEC College of Engineering and Technology, Greater Noida -201306, India*
[e] *Department of Botany, Meerut College, Meerut-250001, India.*
[f] *Department of Biotechnology, MLN National Institute of Technology, Allahabad-211004, India.*

Abstract

The aim of this study was to undertake an assessment of the groundwater quality near city drainages of the Meerut region. Groundwater samples collected from different locations in winter and summer at increasing distances (5 to 70 m) from the drainages were assessed for their suitability for human consumption. The samples were analyzed for various bacteriological parameters including total viable count (TVC), total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). Additionally, physico-chemical [pH, dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD)] were assessed. Heavy metals like Al^{3+} was detected in 83% and Cd, Cu, Zn in 75% and Pb in 41% water samples in winter while during summer season the percentage was slightly higher. Total viable as well as coliforms count exceeded the maximum permissible limits in most water samples irrespective of distance from drainages. The higher most probable number (MPN) values and presence of antibiotic resistant faecal coliforms and streptococci in the water samples suggest the presence of pathogenic microorganisms, heavy metals as well as organic load decreased with increase in distance.

Keywords: coliforms; groundwater; heavy metals; microbiological quality; physico-chemical quality

1. Introduction

Meerut city spans (29° 01' N, 77° 45' E), with a population of about 3.4 millions. The drainages in Meerut city, which cross the whole of the city, are treated like an open drain by the citizens who discharge raw sewage, industrial waste and garbage unchecked. The organic waste, sludge and garbage dumping has reduced the carrying capacity of the drainages, once considered as excess rain water drainers of the city. India is rich in ground water resources, nearly 80% of rural residents rely on untreated ground water for potable water. It is currently threatened by a combination of over-abstraction and microbiological and chemical contamination. Harmless heterotrophic microorganisms occur, such as *Flavobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., *Chromobacterium, Chromobacter* spp. and *Alcaligenes* spp.

Microorganisms are widely distributed in nature, and their abundance and diversity may be used as an indicator for the suitability of water (Okpokwasili and Akujobi, 1996). A wide range of pathogenic microorganisms can be transmitted to humans via water contaminated with faecal material. These bacteria include enteropathogenic agents such as salmonellas, shigellas, enteroviruses, and multicellular parasites as well as opportunistic pathogens like *Pseudomonas aeroginosa, Klebsiella, Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Hodegkiss, 1988). It is not practicable to test water for all these organisms, (Cairneross *et al*., 1990). An indirect approach is based on the assumption that the estimation of groups of normal enteric organisms will indicate the level of faecal contamination of the water supply (WHO, 2000).

Heavy metal contamination in the ground water is a major concern because of their toxicity. Heavy metals such as Al, Cd, Cu, Zn, and Pb are potential soil and water pollutants. The heavy metals entering the ecosystem may lead to geoaccumulation, bioaccumulation and biomagnification (Krishnamurthy and Pushpa, 1995).

Our objective was to investigate the incidence of indicator organisms, coliforms, faecal coliforms and faecal streptococci in relation to physiochemical parameters and heavy metals of ground water samples in Meerut, at different locations during winter and summer seasons.

Table 1. Inhibition zone (mm) of antibiotics in different bacterial strains

Antibiotics	Bacterial isolates				
	Streptococci sp.	*Pseudomonas aeroginosa*	*E. coli*	*Enterobacter* sp.	*Proteus* sp.
Erythromycin (E^{10})	0.65 ± 0.07^{e}	0.60 ± 0.14^{e}	0.25 ± 0.07^{e}	0.45 ± 0.212^{d}	0.60 ± 0.141^{e}
Rifampicin (Rif^{2})	0.65 ± 0.07^{e}	0.65 ± 0.07^{e}	0.20 ± 0.00^{e}	0.10 ± 0.00^{d}	0.40 ± 0.141^{e}
Tetracycline (Tt^{10})	0.80 ± 0.14^{e}	0.60 ± 0.141^{e}	0.30 ± 0.141^{e}	0.65 ± 0.212^{d}	0.70 ± 0.141^{e}
Methicilin (Met^{10})	8.0 ± 1.41^{d}	6.0 ± 1.41^{d}	0.30 ± 0.141^{e}	0.35 ± 0.212^{d}	8.0 ± 0.141^{d}
Ofloxacin (Of^{2})	12.5 ± 0.707^{c}	13.0 ± 1.41^{c}	12.5 ± 0.707^{c}	14.5 ± 3.53^{b}	11.0 ± 0.141^{c}
Ampicillin (Am^{10})	13.0 ± 1.41^{c}	14.0 ± 1.41^{b}	9.0 ± 1.414^{d}	14.0 ± 2.82^{b}	12.5 ± 0.707^{b}
Ciprofloxacin (Cip^{10})	14.5 ± 0.707^{b}	12.5 ± 0.707^{c}	14.0 ± 1.41^{b}	17.0±1.41a	$14.0\pm.0141^{b}$
Gentamycin (Gen^{10})	15 ± 0.141^{a}	13.0 ± 1.41^{c}	14.0 ± 0.141^{b}	12.5 ± 0.707^{c}	11.0 ± 0.141^{c}
Neomycin (N^{30})	16 ± 1.41^{a}	15.5 ± 0.70^{b}	17.5 ± 2.12^{a}	15.5 ± 2.12^{a}	15.0 ± 0.141^{b}
Gentamycin (Gen^{30})	17.5 ± 2.12^{a}	18.5 ± 0.70^{a}	16.0 ± 0.00^{a}	19.0 ± 0.00^{a}	18.0 ± 0.141^{a}

Diameter of zone of inhibition is a mean of duplicate ± SD (mm). Differences were assumed statistically using one way ANOVA followed by Duncan's test *p≤0.05*. Means followed by the same letter(s) (a, b, c, d, e) are not significantly different at *p = 0.05* with in the column.

2. Materials and Methods

2.1. Study area

Twelve groundwater samples were obtained from different locations of Meerut city. Sam ples were collected from different hand pumps (India Mark II), at increasing distances (5-70 m) from the city drainages during winter and summer seasons 2012. The samples were taken from depths of 20-35 m (UPJN 2012), collected in sterile glass Schott bottles (1 litre) triplicate from each site were transported in ice boxes at 3°C to the laboratory for analysis (Sati *et al*., 2011).

2.2. Bacterial analysis

The bacterial population (total viable count, TVC), coliforms and faecal streptococci were detected as described by (APHA *et al*., 1999). Antibiotic disc sensitivity test of isolated pure bacterial cultures was done as described by Kumar (2011). The isolated bacterial strains were characterized as described in Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994) and API 20E kit (Biomerieux, USA).

2.3. Physico-chemical analysis

Physicochemical parameters, including pH, were analyzed on site at the time of sample collection by a water analysis kit (Model LT-61, Labtronics, Guelph, Ontario, Canada). Other parameters i.e. DO, BOD and COD were analyzed by a titrimetric method (APHA *et al*., 1999). The water samples were analysed for the presence of Al^{3+}, Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{1+} using the Buck Scientific 210VGP Atomic Absorption Spectrophotometer. ANOVA was carried out to find out significance at 5% levels. In figures, error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker.

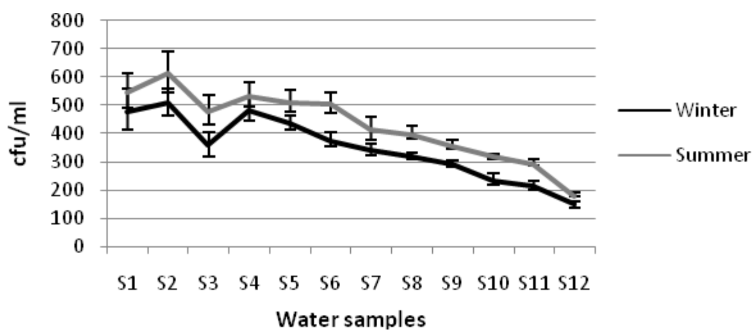

Figure 1. Total viable count (TVC) in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

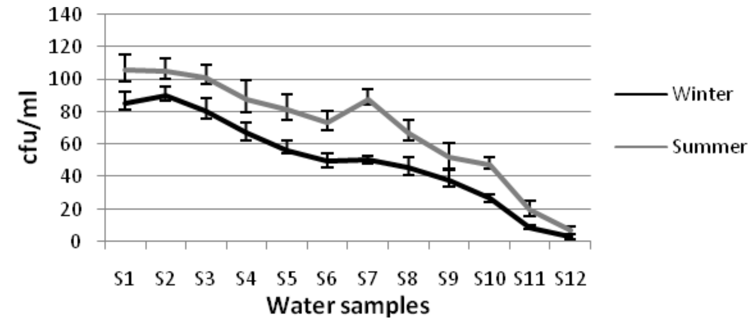

Figure 2. Total coliforms (TC) in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

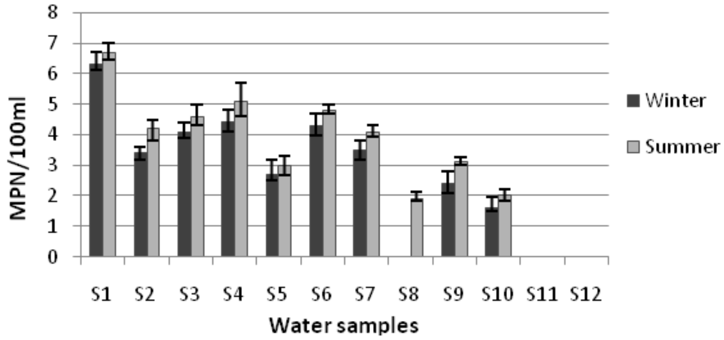

Figure 3. Faecal streptococci (FS) in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

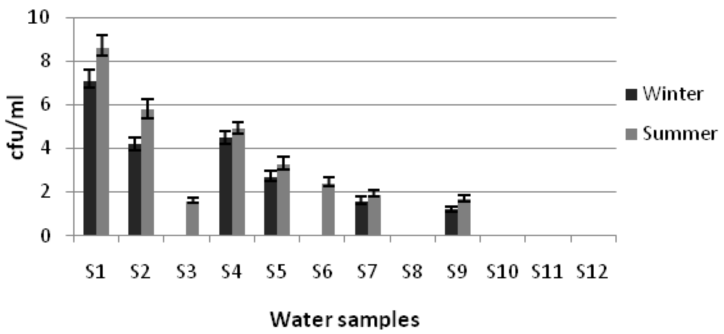

Figure 4. Faecal coliforms in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

3. Results

The TVC count in water samples decreased with increase in distance from the drainage. Slight increase was observed in samples S2 and S4 compared in S1 and S3 in both seasons, other samples S5 to S12 showed a decreasing trend as distance from the drainages increased. Highest TVC count during winter was observed in sample S2 (510 cfu/ml), followed by S4 (483 cfu/ml) and minimum in S12 (153 cfu/ml). Highest TVC during summer in S1 (546 cfu/ml) and the smallest in S12 (180 cfu/ml) (Fig. 1). During winter, highest TC count was observed in S2 (90.3 cfu/ml), followed by S1 (85.5 cfu/ml), and minimum in S12 (2.7 cfu/ml), while in summer highest count was in S1 (106.1 cfu/ml) and lowest in S12 (6.7 cfu/ml) (Fig. 2).

Highest FS count was observed in S1 (6.3 cfu/ml), and minimum in S10 (1.6 cfu/ml), while S8, S11 and S12 did not show any FS in winter season. The FS count in summer was highest in S1 (6.7 cfu/ml) and lowest in S10 (3.1 cfu/ml). In summer, FS count was observed in S8 (1.9 cfu/ml) (Fig. 3). The FC in samples S3, S6 and S8 exhibited no count, while S2, S5 and S7 exhibited FC values, (Fig. 4).

The MPN count in all samples also decreased with the distance from the drainage. Highest MPN count was observed during winter in sample S1 (1800/100 ml), followed by S2 (1150/100 ml), S3 (840/100 ml) and minimum in S12 (16/100 ml). Summer season also showed similar tendency, highest MPN (1800/100 ml) in S1 and lowest in S12 (25/100 ml) (Fig. 5).

A total of 517 bacterial strains from the ground water samples were isolated, out of which 272 isolates including coliforms and non coliforms were identified up to genus level. *E. coli* was found to be the most dominant with an occurrence percentage of 23.5% followed by *Pseudomonas* (14.3%) and *Bacillus* (13.9%) (Fig. 6). Among the tested drugs rifampicin (Rif^{2}) and tetracycline (Tc^{10}) were least effective, while gentamycin (Gen^{30}), neomycin (N^{30}) and Ciprofloxacin (Cip^{10}) exhibited highest zone of inhibition (Table 1).

The pH values ranged from minimum 6.8 (samples S1 and S8) to maximum 7.53 (S 6 and S10) (Fig. 7). In general DO content of all the samples showed a uniform trend of increase with increase in distance from drainages (Fig. 8). Minimum DO was observed in sample S1 (6.4/100 ml) while the maximum was found in S12 (24.6/100 ml) in winter, while in summer the values ranged from (5.8 to 20.6 mg/L).

Highest BOD value in winter was observed in sample S1 (12.9/100 ml) while the least found in S12 (1.7/100 ml). Water sample S8 exhibited BOD (5.4/100 ml), it was slightly unusual as sample S7 showed less BOD (4.9/100 ml). In summer, BOD ranged from 2.4 mg/L in sample S12 to 15.6 mg/L in sample S1 (Fig. 9). The COD values of most of the samples were above the permissible limits (Fig. 10). Highest COD value in winter was observed in sample S1 (15.3/100 ml) while the least found in S12 (1.6/100 ml).

In ground water samples, Al was detected in 83% samples; Cd, Cu, Zn in 75% and Pb were found in 41% samples in winter while during summer the percentage was slightly higher (Fig. 11), while the amount of heavy metals were slightly higher during summer (Fig. 12).

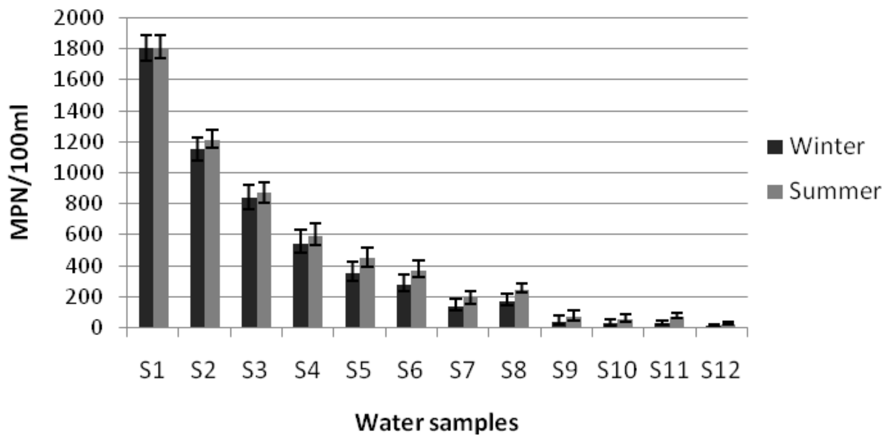

Figure 5. Most probable number (MPN) count in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

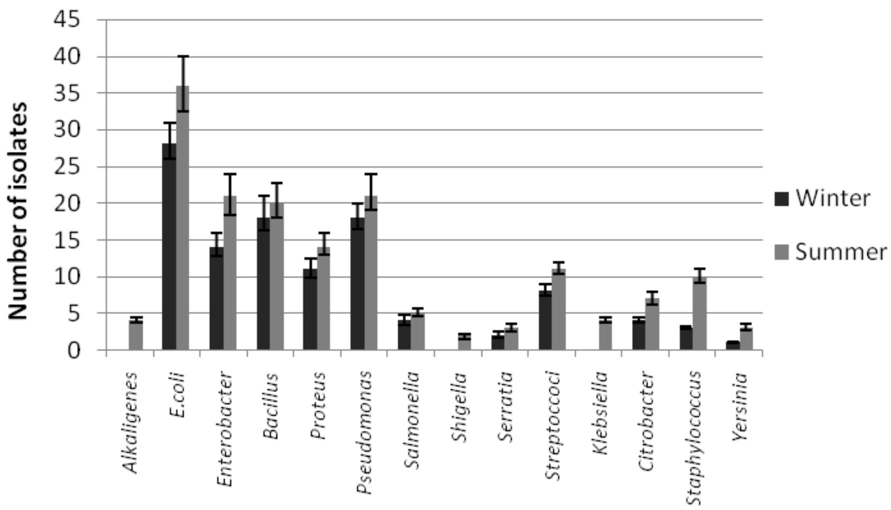

Figure 6. Seasonal variation of prevalant genera in ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

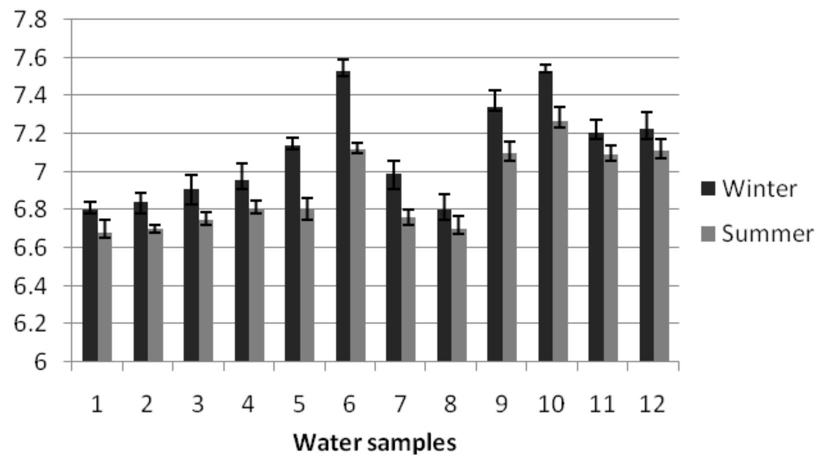

Figure 7. pH of ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

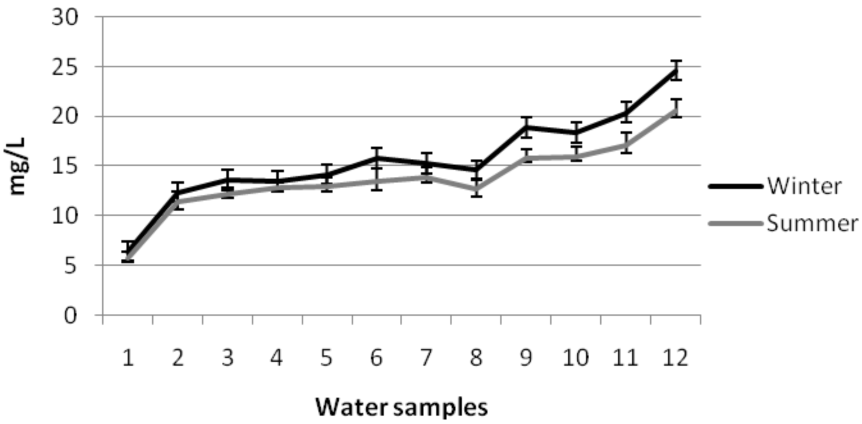

Figure 8. Dissolved oxygen (DO) of ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

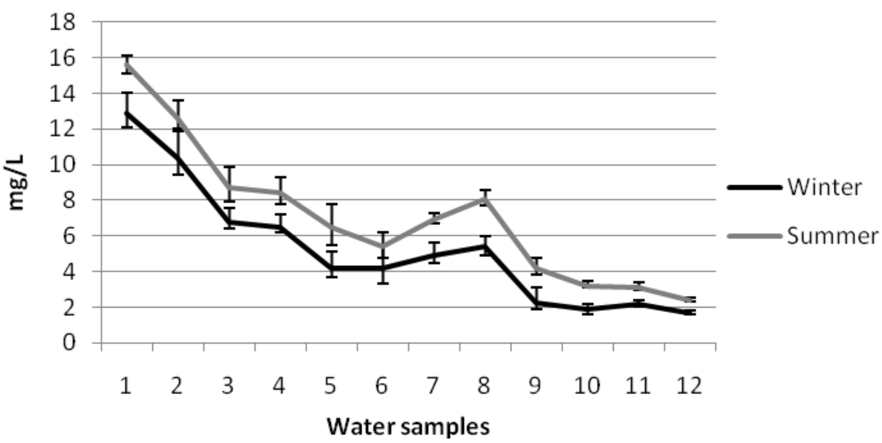

Figure 9. Biological oxygen demand (BOD) of ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

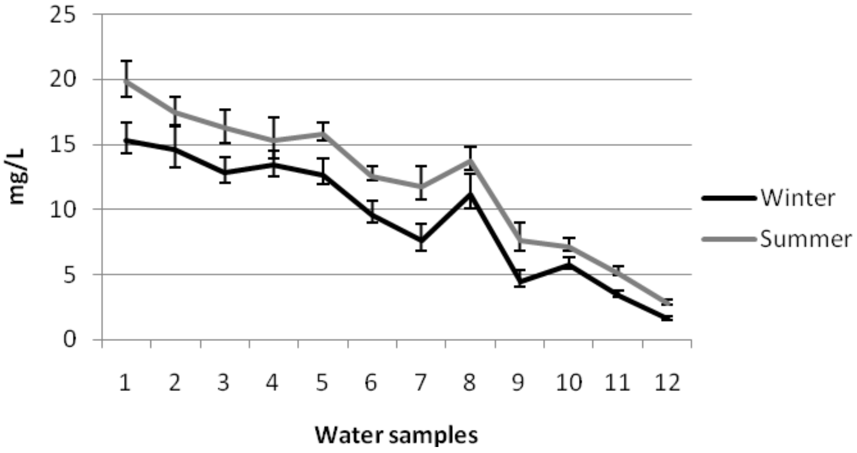

Figure 10. Chemical oxygen demand (COD) of ground water samples (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

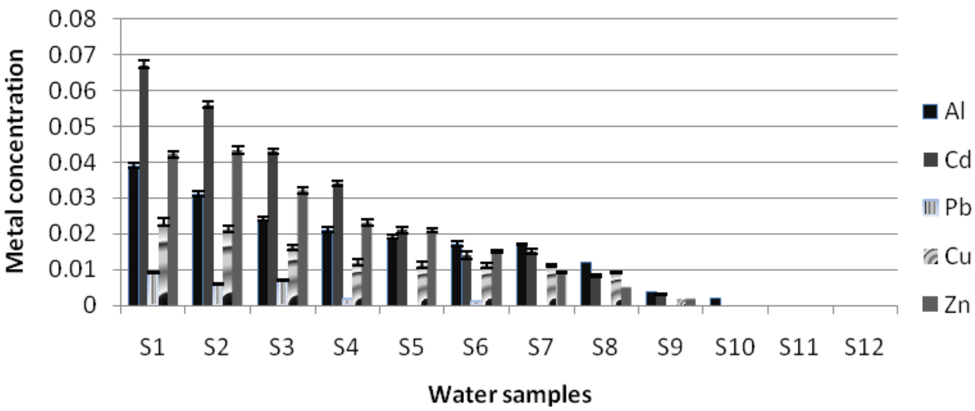

Figure 11. Heavy metals (ppm) in ground water samples during winter season (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

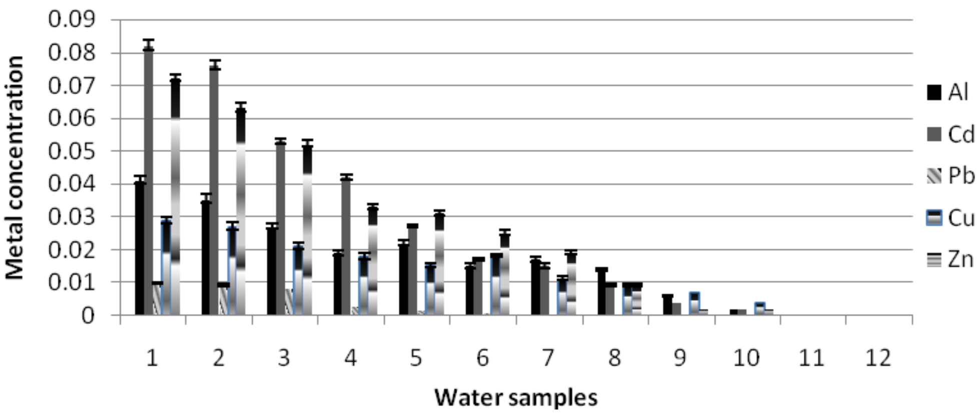

Figure 12. Heavy metals (ppm) in ground water samples during summer season (Error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker)

4. Discussion

It has been generally believed in India that groundwater is relatively free of microorganisms, therefore, fit for human consumption without treatment. However, our results show clearly that all samples of hand-pump water examined, contained high counts of bacteria and high level of physico-chemical pollutants.

Thus, the MPN, TVC, TC, FC and FS, counts were high, as per the (WHO, 2000) standards, in all the water samples collected from the hand pumps. The bacterial load decreased in samples with increasing distances from the drainages, indicating the direct effect of seepage from the drainages to the ground water level. As the distance increased, level of contamination also decreased, due to soil strata that act as filtering material. All the water samples exhibited a regular trend of decreasing microbial load as the distance increased from the drainages, except the water sample S8, which showed slight higher values. This could be due to contamination of ground water with any polluting channel or leakage from domestic sewage line, improper construction, animal wastes, proximity to toilet facilities, sewage, refuse dump sites, and various human activities around the hand pump (Bitton, 1994) in that area. In summer, the samples revealed FS count in S8 and FC in samples S3 and S6. The FS was not detected in sample S8 in winter and FC were not observed in S3, S6 and S8, which may be because of their less number compared to TVC and TC and due to this these bacterial strains were not able to cross the soil strata barrier. The reasons for the high number of total coliforms were due to the discharge of human and animal faeces into the water bodies. Isolation of pathogenic and potentially pathogenic microorganisms such as *E. coli, Streptococci* sp., *Proteus* sp. *Enterobacter* sp. and *Pseudomonas aeruginosa* is of highly importance and indicated that ground water is unsafe (EPA, 2003).

Comparison of bacteriological and physico-chemical parameters clearly indicates that higher the organic load (BOD and COD) the higher was the bacterial count. Moreover, this relationship was also observed in both seasons, but bacterial counts as well as physico-chemical parameters were higher in summer; this could be due higher temperature. Sah *et al*. (2000) have stressed that the pollution in rivers and water bodies from industries may adversely affect aquatic life of water bodies as well human health in the vicinity of rivers/lakes.

Several pathogenic genera were isolated and identified. Many of these genera including *Enterobacter, Proteus* and *Staphylococcus* etc. are found associated to human activities. Dominance of these bacterial genera in ground water suggests that they may be explored as indicators. A high count of *E. coli* in ground water samples indicates the potential presence of pathogenic microbes of intestinal origin but gives no indication of the sources of microbial pollution. Other genera were also obtained which, although were less abundant, still pose a threat if water is consumed untreated. Isolation of such potential pathogens from ground water shows that the situation with respect to water quality is alarming in Meerut city region. Similarly, (Mclellan *et al*., 2001) stated that faecal pollution indicator organisms can be used to a number of conditions related to the health of aquatic ecosystems and to the potential for health effects among individuals using aquatic environments.

The high coliform count obtained in the samples may indicate that the water sources are faecally contaminated (EPA, 2003; Krishnan *et al*., 2007). The antibiotic resistivity pattern of isolated bacterial strains indicates that resistant types of pathogenic strains were present in ground water samples. These antibiotic resistant strains could be a threat to consumers in two ways, first they are resistant to antibacterial drugs and secondly they may transfer their genome to sensitive strains, thus increasing the chance of drug resistant strains.

The pH of all the water samples were in agreement with EPA as the standard pH of water ranged from 6.5-8.5 (EPA, 2003), all our water samples tested falls within the range. The level of the oxygen in water (5.8 to 24.6 mg/L) is an indicator of healthy state of water and values below 3 mg/L are hazardous to human. The DO values in the samples collected were more than 3 mg/L (BIS, 1993). The reason for the low DO content in sample S1 (5.8 mg/L) in summer was due to high decomposition of organic matter, which indicates a high pollution load in the water, compared to other samples (Sharma *et al*., 2010). Water with BOD levels <4 mg/L are deemed as clean, while those >10 mg/L are considered polluted and unsafe (BIS, 1993). This study reported that water samples S1 to S8 were having a high organic load as the BOD level was very high (3.2 to 15.6 mg/L). COD values should be <10 mg/L at the end of treatment of water. In our case the COD varied from 1.6 mg/L (S12 in winter) to 19.8 mg/L (S1, in summer), therefore, water samples S1 to S6 and S8 were polluted.

For human health protection, guidelines for the limits of heavy metals in water samples have been set by EPA, European Union Commission, USEPA, WHO (Marcovecchio *et al*., 2007). Aluminium, Cadmium and Lead have maximum contaminant levels of 0.2, 0.003 and 0.01 PPM respectively (WHO, 2000). Aluminium toxicity is associated with continuous low level exposure, this can eventually lead to serious health effects and also a possible link between Aluminium in drinking water and dementia, moreover Aluminium contamination may cause Alzheimer's disease (Momodu and Anyakora, 2010). Higher concentrations of Cadmium are carcinogenic; it may cause acute health effects and kidney disease (Orisakwe *et al*., 2006). Higher concentrations of lead may lead to general metabolic poisoning; it is a neurotoxic and causes human metal toxicosis (Zeitz *et al*., 2007). The ground water quality standard of Zinc desirable limit is 5.0 mg/L and maximum permissible limit is 10.0 mg/L (WHO, 2000). The ground water quality standard of Copper desirable limit is 0.05 mg/L and the maximum permissible limit is 1.5 mg/L (WHO, 2000). In all the ground water samples tested, Al and Cd were above the limits in some water samples, while Cu, Pb and Zn were within the limits of all the ground water samples (BIS, 1993; WHO, 2000).

5. Conclusions

Conclusively, proper hand pump location and cemented construction of drainages, control of human activities to prevent sewage from entering water body are the keys to avoid bacterial and heavy metal contamination of drinking water. Water borne diseases are due to improper disposal of refuse, contamination by sewage, surface runoff, therefore programmes must be organized to educate the general populace on the proper disposal of refuse, treatment of sewage and the need to purify our water to make it fit for drinking because the associable organisms and heavy metals are of public health significance. In areas lacking in tap water as in rural dwelling, educative programmes must be organized by researchers and government agencies to enlighten the residents on the proper use of ground water.

Acknowledgments

The authors are grateful to the Management, College of Applied Education & Health sciences, Meerut, India for providing research facilities to carry out this work.

References

APHA, AWWA, WEF. 1999. Standards for Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington DC USA 1990.

BIS (Bureau of Indian Standard). Indian standard specification for drinking water. IS: 10500, Indian Standard Institute 1993.

Bitton G. Waste Water Microbiology. Gainesville, New York Wiley- Liss. 1994,.118.

Cairneross S, Carruthers I, Curtis D, Feachem R, Bradley D, Baldwin G. Evaluation for Village Water Supply Planning. Wiley, Chichester, 1990, 277.

EPA (Environment Protection Agency). US Environmental Protection Agency Safe Drinking Water Act. EPA 816 – F – 03 –016 2003.

Hodegkiss IJ. Bacteriological monitoring of Hong Kong marine water quality. Environment International 1988; 14: 495-499.

Holt JG, Kreig NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology, Ninth ed. Williams and Wilkins, Baltimore, MD, 1994, 151-57.

Krishnamurthy CR, Pushpa V. Toxic metals in the Indian Environment. Tata McGraw Hill Publishing Co. Ltd., New Delhi. 1995, 280.

Krishnan RR, Dharmaraj K, Kumari BDR. A comparative study on the physicochemical and bacterial analysis of drinking, borewell and sewage water in the three different places of Sivakasi. Journal of Environmental Biology 2007; 28: 105-08.

Kumar V. Laboratory Manual of Microbiology. Scientific Publishers, Jodhpur, India, 2011, 132-34.

Marcovecchio JE, Botte SE, Freije RH. Heavy metals, Major metals, Trace elements. *In*: Handbook of Water Analysis. (*Ed*: Nollet LM) 2nd ed. London, CRC Press, 2007, 270-311.

Mclellan SL, Daniels AD, Salmore AK. Clonal populations of thermotolerant enterobacteriaceaes in recreational water and their potential interference with fecal Escherichia coli count. Applied and Environmental Microbiology 2001; 67: 4934-38.

Momodu MA, Anyakora CA. Heavy Metal Contamination of Ground Water: The Surulere Case Study: Research Journal of Environmental and Earth Sciences 2010; 2(1): 39-43.

Okpokwasili GC, Akujobi TC. Bacteriological indicators of tropical water quality. Environment Toxicity and Water Quality 1996; 11: 77-81.

Orisakwe OE, Igwilo IO, Afonne OJ, Maduabuchi JU, Obi E, Nduka J C. Heavy metal hazards of sachet water in Nigeria. Archives of Environmental and Occupational Health 2006; 61(5): 209-13.

Sah JP, Sah SK, Acharya P, Pant D, Lance VA. Assessment of water pollution in the Narayani River, Nepal. International Journal of Ecology and Environmental Science 2000; 26: 235-52.

Sati A, Sood A, Sharma S, Bisht S, Kumar V. Bacterial indicators of faecal pollution and physiochemical assessment of tributaries of Ganges River in Garhwal Himalayas, India. RMZ-Materials and Geoenvironment 2011; 58:129-42.

Sharma P, Sood A, Sharma S, Bisht S, Kumar V, Pandey P, Gusain M, Gusain OP. Bacterial indicators of faecal pollution and physiochemical assessment of important North Indian lakes. RMZ-Materials and Geoenvironment 2010; 57: 25-40.

UPJN (Uttar Pradesh Jal Nigam). http://www.upjn.org/rural.aspx/ http://www.upjn.org/index.aspx 2012.

WHO. Hazardous chemicals in human and environmental health: A Resource Book for School, College and University Students. World Health Organization, Geneva 2000.

Zietz BP, Lap J, Suchenwirth R. Assessment and management of tap water Lead contamination in Lower Saxon, Germany. International Journal of Environment and Health Research 2007; 17(6): 407-18.

Received 4 August 2012
Accepted 18 September 2012

Correspondence to
Dr. Vivek Kumar
Department of Microbiology,
Division of Biosciences,
College of Applied Education & Health Sciences,
Meerut-250003,
India.
Emal: vivek@caehs.edu.in