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ASSESSMENT OF MICROBIOLOGICAL CHARACTERISTICS OF LENTIC WATER BODIES WITH RELIGIOUS ACTIVITIES: A MULTI-LOCATION STUDY IN HARYANA, INDIA

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ABSTRACT: The present study was undertaken to evaluate the impact of religious activities on the water quality of eight prominent water bodies of Haryana, India in terms of total bacterial count and Most Probable Number (MPN) of faecal coliforms. Significant ($P < 0.05$) increase in Standard Plate Count (SPC) of bacteria population was observed at most of the sites after religious activities. In season wise comparison SPC was high at sites 4 and 8during summer where at sites 2, 5, 7 during monsoon followed by at sites 1, 3, 6 during the post monsoon. Overall SPC was higher during monsoon season followed by summers than in post-monsoon and low in winters. The results suggested that the values of MPN count of coliform bacteria significantly ($P < 0.05$) increased at all the eight selected water bodies after the religious activities and it was exceeding the limits as prescribed by water quality standards for mass bathing. The higher increase was noticed at the sites where mass bathing was more prominent. MPN index was also recorded high during monsoon season and summer followed by winters and post monsoon season. The statistical analysis revealed that the distribution of coliform bacteria was remarkably influenced by total bacteria count as a positive correlation was observed in SPC and MPN count of bacteria ($r = 0.389$, $P < 0.05$). The results of this study revealed the significant correlation between bacterial count and the pollution indicating water quality characteristics *viz.*, BOD ($r = 0.254$, $P < 0.05$), the CO_2 ($r = 0.635$, $P < 0.01$), NO_3^- ($r = 0.728$, $P < 0.01$), PO_4^- ($r = 0.713$, $P < 0.01$) and with ammonia ($r = 0.897$, $P < 0.01$) respectively.

Key words: Bacteria, Coliform, Physico-chemical, Religious activities, Water quality

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INTRODUCTION

Water is the basic and prime necessity of all living organisms; is essential part of protoplasm and creates a state for metabolic activities to occur smoothly (Dubey and Maheshwari, 2006). But in today's scenario the water quality is being deteriorated by various types of anthropogenic activities threatening the natural productivity of target system, the biodiversity, the state of fisheries, aesthetic qualities of the natural ecosystem and also human health. Globally it has been estimated that more than 250 million cases of waterborne diseases reported each year, which results about 10 million deaths (Esrey and Habicht, 1985). Among the anthropogenic activities, mass bathing or ritual activities such as bathing, washing and other rituals adds detergents, soap, ash, polythene carry bags and other domestic wastes in water increases the pollution load on the water body (Bhatnagar and Sangwan, 2009; Marale *et al*., 2010; *et al*., 2011; Arora *et al*., 2013) by enhancing faecal coliform load (Kushreshtha and Sharma, 2006; Telang *et al*., 2009; Zabed *et al*., 2014).

During festivals people used to take holy dip in water and also use it for drinking (Aachman), irrespective of its water quality (Semwal and Alkolkar, 2006).The probability of ingesting infective dose of disease causing microorganisms is very high considering that the fact that water borne pathogens generally have low infective dose so, there is a risk of cholera, typhoid, dysentery, rashes, leptospirosis and more, that affect human health. Bacteria have a keyposition in global ecosystems which determines the quality of water and are the fate determinators of environmental pollution and the bacteria of coliform group are considered the primary indicators of faecal contamination (Raina *et al.,* 1999) that have been correlated with the incidence of gastrointestinal disorders (Morace and McKenzie, 2002).The link between poor microbiological water quality and infectious diarrhea is well established (Do *et al*. 2007; Oswald *et al*., 2007; Cronin *et al*. 2008).

Haryana state in India, where religious activities are carried out in prominent lentic water bodies regularly during occasions like solar eclipse, amavasya (new moon day), Shradh period (up to 15 days during Sep. / Oct.). About 10 millions of people took holy bath and perform their rituals during such occasions. Since, bathing water quality is essential for bather's health as well indicates the overall status of inland water bodies therefore, regular monitoring of water bodies is prerequisite for physico-chemical and bacteriological analysis with reference to the quality of water not only to check the outbreak of diseases and occurrence of hazards but also to prevent the water from further deterioration. Therefore, the present study was conducted to assess the water quality in terms of total bacterial count and coliform count. An attempt has also been made to find the correlation of physico-chemical characteristics with bacteriological parameters to evaluate the water quality.

MATERIALS AND METHODS

Study area

A total of eight prominent water bodies in four district of Haryana were selected for the studies that were being used for the mass bathing and other religious activities (Table 1).

Sample collection

Water samples for bacteriological analysis were collected two days before every religious activity and one day after religious activity from selected stations, in triplicate, in sterile test tubes packed in ice box and also seasonally during Summer, Monsoon, Post monsoon and Winter from February 2012 to March 2013 for physico-chemical and bacteriological analysis. During the study period the religious activities were performed two times at stations Ban Ganga tirth (site 3), Brahmsarovar(site 4), Jyotisar (site 5), Saraswati tank (site 6) and Pandu-Pindara tirth (site 8); one time at stations Kapalmochan (site 1), Kulotaran tirth (site 2) and Phalgu tirth (site 7).

Microbiological analysis

Standard techniques were followed for collection and bacteriological analysis of water samples (APHA, 2005). The most probable number (MPN) method was employed for the total and faecal coliforms.

1. **Standard Plate Count (SPC)**

   The water sample were inoculated on standard Nutrient Agar Media at various dilutions and incubated for 24 to 48 hours. After Incubation, the total numbers of colonies per plate were counted and it was multiplied by the dilution factor and total population was expressed as Colony Forming Units / ml (CFU/ml) of water sample.

2. **Method for Testing Total Coliform and *E.Coli***

   Multitube fermentation technique / MPN method

   Concentration of total faecal coliform bacteria was reported as Most Probable Number per 100 ml (MPN / 100 ml) following standard procedures (APHA, 2005).

   I. **Presumption test:**
   Double strength (2X) and single strength (1X) MacConkey broth containing upside-down Durham tubes inoculated with water sample. Positive tubes showing gas production or colour change of the broth from violet to yellow (contain bubble in Durham tube) were recorded.

   II. **Conformation test for total coliform:**
   1 ml of inoculum from the positive tube from presumption test was inoculated in Brilliant green broth. Number of tubes positive for gas production (contain bubble in Durham tube) were recorded.

   III. **Completed test for *E. coli*:**
   The inoculum from positive tubes of confirmed test was inoculated on EMB (Eosin Methylene Blue) agar media. Presence of metallic green colonies confirmed the presence of *E. coli* colonies.

Physico-chemical characteristics

Physico-chemical characteristics and were analyzed following standard procedures (APHA, 2005 and Garg*et al*., 2002) for parameters viz. Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammonia, ortho-phosphate and free Carbon dioxide (CO_2).

RESULTS AND DISCUSSION

Microorganisms are generally used as an indicator for the assessment of drinking water and bathing water quality due to their wide distribution, abundance and diversification in nature (Okpokwasili and Akujobi, 1996; Baghel *et al*., 2005).Measuring of indicator organismsin waters is comparatively cheaper, easier and more common methods than measuring the particular pathogens directly. However, indicator organisms used during analysis of water quality have a limitation in their inability to predict pathogen presence and health risks (Russell *et al.*, 2007).The presence of coliform bacteria can be regarded as an important indicator of faecal contamination for water bodies. Various ritual activities like addition of urine, soap, detergents, floral offerings, flour, oil etc. in the water bodies, tend to increase the value of BOD, COD, bacterial populations and faecal coliform (Vyas *et al.*, 2006; Kumar *et al*., 2010; Gupta *et al.*, 2011). In the present studies significant ($P < 0.05$) increase in Standard Plate Count (SPC) of bacteria population was observed at most of the sites (Fig. 1) i.e. Brahmsarovar, Phalgu, Pehowa, Ban-Ganga, Jyotisar, Pindara after the religious activities. A probable reason behind this may be the addition of organic materials and faecal waste due to mass bathing of millions of pilgrims and the religious activities performed by them, supporting the earlier findings of Sood *et al*., 2008; Arora *et al.*, 2013; Zabed *et al*., 2014. Out of all selected sites significantly ($P<0.05$) high SPC of bacterial population was reported at site Phalgu followed by Kirmach, Pindara and Pehowa. Overall average low SPC was observed at site Brahmsarovar indicating less pollution status of the water body. It may be due to large size of the water body and not having the cemented floor. So, as a result of such mass bathing or religious activities there was significant increase in bacteriological contamination of waters, thus deteriorating the water quality and hence there would be high risks ofgetting illnesses when bathing in such waters.

When season wise comparison was done the SPC of bacterial population was high during summer and the monsoon season followed by in post monsoon season and it was low during the winter season (Table 2). Higher SPC in summer and monsoon might be due to the higher temperature and high humidity favoring the growth of bacterial population during these months.Similar to these findings maximum number of total coliform was reported during summer by Agarwal and Razwar (2010); Krishna *et al.* (2012) and minimum number of total coliform during winters was reported by Agarwal and Razwar (2010).

Presence of faecal contaminations in water revealed the potential presence of pathogenic enteric microorganisms that are recognized to be the major cause of waterborne diseases. Among enteric organisms *Enterobacter* sp., *Salmonella* sp., *Klebsiella* sp. and *E. coli* etc. are the most common bacteria leading to outbreak of intestinal disorders (Krishna *et al*., 2012). In the present studies, Most Probable Number of fecal bacteria population (MPN Index / ml) was calculated before and after the religious activities. Although increase in MPN Index of faecal bacteria population was recorded at all the site after rituals and mass bathing of the huge gathering of millions of people (Fig. 2) but MPN/ 100 ml of water sample was found to be significantly high at site Pindara (1255±202) after solar eclipse day (June 20, 2012); at Phalgu (1255±202) and Pehowa (2000±400) after the "Shradh" period (2 to 15 October, 2015); Ban-Ganga (725±108) after the Vaishakhi fair (April 13, 2012) and further at Kapalmochan (1255±202) it was found to be significantly increased after the fair (November 26 to 29, 2012) held during the occasion of Gurunanak jayanti (Fig. 2). Significant increase in MPN Index was may be due to high level of pollution caused in the form of immersion of flour, floral offerings, bathing, urination etc. during these peak bathing dates. The various studies revealed that the organic materials from a wide range of sources support bacterial populations, a portion of which are capable of responding positively to the Total Coliform and faecal Coliform (Ajayan *et al*., 2015). Patra *et al* (2009 showed a positive relationship between faecal indicators and pathogenic microorganisms. Increased bacterial count in surface water due to ritual activities and mass bathing were also reported by Semwal and Akolkar (2006); Arora *et al*. (2013) and Zabed *et al.* (2014). When MPN index value of all the sites before and after the religious activity were compared with the values of water quality guidelines (total coliform count should not be more than 500 in terms of MPN per 100 ml of water sample) suggested by Tyagi*et al.* (2013). It was reported that after the religious activities Pindara, Ban-Ganga, Phalgu, Kapalmochan and Pehowa were having the MPN Index value higher than the values except Brahmsarovar and Kirmach sites (Fig. 2) indicating that water was not suitable for bathing at sites 1, 3, 5, 6, 7, 8 after the religious rituals as there may be higher the incidence of gastroenteritis and other transmissible diseases. In German freshwater epidemiological study (Wiedenmann *et al.* 2002) *E. coli* has been found to be linked to swimmers' illness risk. The Most Probable Number for the estimation of coliform population was calculated and compared season wise by using ANOVA followed by Duncan's Multiple Range test (Table 3). MPN Index (MPN / 100 ml) at Kapalmochan was significantly high ($P< 0.05$) during summer may be high due to high evaporation rate and low water level. Maipa *et al*. (2001) also reported increased *E. coli* and fecal coliforms during the summer period. Whereas, at Pehowa, Brahmsarovar, Banganga, Jyotisar, Kirmach it was significantly ($P < 0.05$) high during monsoon season, may be due to inflow of water inside the water bodies from various point and non-point sources. One of the possible reasons of higher count in monsoon season may be higher humidity that helped increasing in settling down of airborne bacteria (Arundel *et al*., 1986).

According to Javed *et al.* (2014) values of total coliform significantly higher in monsoon may be attributed due to entry of more pathogens in surface water when flood flush effectively treated sewage and manure. It was recorded low during winters and post monsoon season. Similar seasonal trend of higher bacterial count during rainy season followed by summer and low in winter was reported by Zabed *et al.* (2014). Bhattarai *et al.* (2008) have also reported low values of MPN during post monsoon and winters supporting the present results. The seasonal MPN Index of coliform at all the sites was compared with water quality guidelines (Table 3) it was higher at site Kapalmochan (summer) and also at Pehowa during monsoon season. Similar results of increase in microbiological pollution in summers concerned with mainly *E. coli* and fecal coliforms were also reported by Maipa *et al.* (2001). Rest of the water bodies had the values of MPN index within the limits of mass bathing in all the seasons, indicating that water quality status of these water bodies was suitable for mass bathing.

Table 1: Details of sites selected and related religious activities taking place there.

S.N.	Name of water body	District	Religious activities
1.	Kapalmochan	Yamunanagar	Purnima (Kartik / Nov) Gurunanakjayanti (Mass bathing)
2.	Brahmsarovar	Kurukshetra	Amavasya, Solar eclipse (Mass bathing)
3.	Jyotisar	Kurukshetra	Amavasya, Solar eclipse (Mass bathing)
4.	Banganga tirth	Kurukshetra	Vaishakhi (April) (Mass bathing)
5.	Kulotaran tirth	Kurukshetra	Fair at September month (Mass bathing)
6.	Saraswati tirth	Pehowa, Kurukshetra	Pind-dan and bathing during Sharad, Solar eclipse and Kartik amavasya
7.	Phalgu tirth	Kaithal	Pind-dan and bathing during Somvati amavasya of Shrad (Sept-Oct)
8.	Pandu-Pindara tirth	Jind	Pind-dan and bathing during Somvati amavasya, Solar eclipse, Sharad

Table 2: Season-wise Standard plate count (CFU/ml) of bacterial population.

	Summer (CFU/ml)	Monsoon (CFU/ml)	Post monsoon (CFU/ml)	Winter (CFU/ml)
Site 1	$1.077\times10^{8} \pm 0.02\times10^{8Cb}$	$40.80\times10^{8} \pm 1.61\times10^{8Db}$	$11360\times10^{8} \pm 277.12\times10^{8Ba}$	$0.53\times10^{8} \pm .0017\times10^{8Cb}$
Site 2	$1444\times10^{8} \pm 13.77\times10^{8Cc}$	$134600\times10^{8} \pm 3527.66\times10^{8Ba}$	$45330\times10^{8} \pm 6489.30\times10^{8Ab}$	-
Site 3	$36.13\times10^{8} \pm 1.41\times10^{8Cb}$	$166.4\times10^{8} \pm 9.73\times10^{8Db}$	$1048\times10^{8} \pm 161.85\times10^{8Ca}$	$0.4027\times10^{8} \pm 0.015\times10^{8Cb}$
Site 4	$262.6\times10^{8} \pm 12.72\times10^{8\,Ca}$	$5.203\times10^{8} \pm 0.02\times10^{8Dc}$	$27.33\times10^{8} \pm 2.18\times10^{8Cb}$	$38.40\times10^{8} \pm 0.92\times10^{8Cb}$
Site 5	$63.73\times10^{8} \pm 2.08\times10^{8Cc}$	$1415\times10^{8} \pm 13.89\times10^{8Da}$	$154.66\times10^{8} \pm 1.5\times10^{8Cb}$	$22.43\times10^{8} \pm 0.32\times10^{8Cd}$
Site 6	$0.805\times10^{8} \pm .0003\times10^{8Cc}$	$30.40\times10^{8} \pm 0.46\times10^{8Db}$	$763.3\times10^{8} \pm 8.81\times10^{8Ca}$	$784\times10^{8} \pm 9.23\times10^{8Aa}$
Site 7	$11730\times10^{8} \pm 162.20\times10^{8Bb}$	$306100\times10^{8} \pm 3733.33\times10^{8Aa}$	$2816\times10^{8} \pm 116.66\times10^{8Cc}$	$1.168\times10^{8} \pm 0.018\times10^{8Cc}$
Site 8	$40400\times10^{8} \pm 4085.74\times10^{8Aa}$	$18830\times10^{8} \pm 556.81\times10^{8Cb}$	$2.11\times10^{8} \pm 0.55\times10^{8Cc}$	$696\times10^{8} \pm 3124099523^{Bc}$

All values are Mean ± S.E of mean

Means with different capital letters in the same column and small letter in the same row are significantly ($p< 0.05$) different (Duncan's Multiple Range test). The first capital letter is denoting the site wise comparison in same season and small letter is denoting only one site comparison during different seasons.

Table 3: Season-wise MPN Index of Coliform bacteria population.

Site name	MPN index /100 ml			
	Summer	**Monsoon**	**Post Monsoon**	**Winter**
Kapalmochan (site 1)	730 ± 190^{Aa}	27 ± 4^{Bb}	41 ± 2.49^{Db}	445 ± 95^{Aab}
Kirmach(site 2)	75 ± 4.49^{Ba}	75 ± 4.49^{Ba}	48 ± 1.5^{CDb}	-
Banganga (site 3)	12 ± 1^{Bc}	32 ± 1^{Ba}	23 ± 0.5^{Eb}	15 ± 1.5^{Cc}
Brahmsarovar (site 4)	21 ± 1^{Bc}	75 ± 4.49^{Ba}	22 ± 1^{Ec}	32 ± 1^{Cb}
Jyotisar (site 5)	25 ± 1.5^{Bc}	173 ± 2.49^{Ba}	67 ± 3.49^{Bb}	175 ± 4.49^{Ba}
Saraswati Tirth (site 6)	215 ± 5^{Bb}	730 ± 189^{Aa}	175 ± 5^{Ab}	31 ± 2^{Cb}
Phalgu (site 7)	48 ± 1.5^{Bb}	17 ± 0.5^{Bc}	48 ± 1.5^{CDb}	348 ± 2.49^{Aa}
Pindara (site 8)	230 ± 10^{Bb}	23 ± 2.49^{Bd}	60 ± 10.5^{BCc}	348 ± 2.49^{Aa}

All values are Mean ± S.E of mean
Means with different capital letters in the same column and small letter in the same row are significantly ($p< 0.05$) different (Duncan's Multiple Range test). The first capital letter is denoting the site wise comparison in same season and small letter is denoting only one site comparison during different seasons.

Table 4: Summary of water quality guidelines for mass bathing / swimming.

S.N	Parameter	MAC Values (Maximum allowable concenteration)
1.	BOD (Mg L^{-1})	≤3 (Tyagi *et al.*, 2013), 6 (Buijs and Toader, 2007)
2.	COD (Mg L^{-1})	15 (Buijs and Toader, 2007)
3.	Nitrate (Mg L^{-1})	5.6 (Buijs and Toader, 2007)
4.	Ammonia (Mg L^{-1})	0.8 (Buijs and Toader, 2007)
5.	o-Phosphate (Mg L^{-1})	0.2 (Buijs and Toader, 2007)
6.	Carbon dioxide (Mg L^{-1})	120 (Buijs and Toader, 2007)
7.	Faecal Coliform (No./100ml of water)	500 (Tyagi *et al.*, 2013)

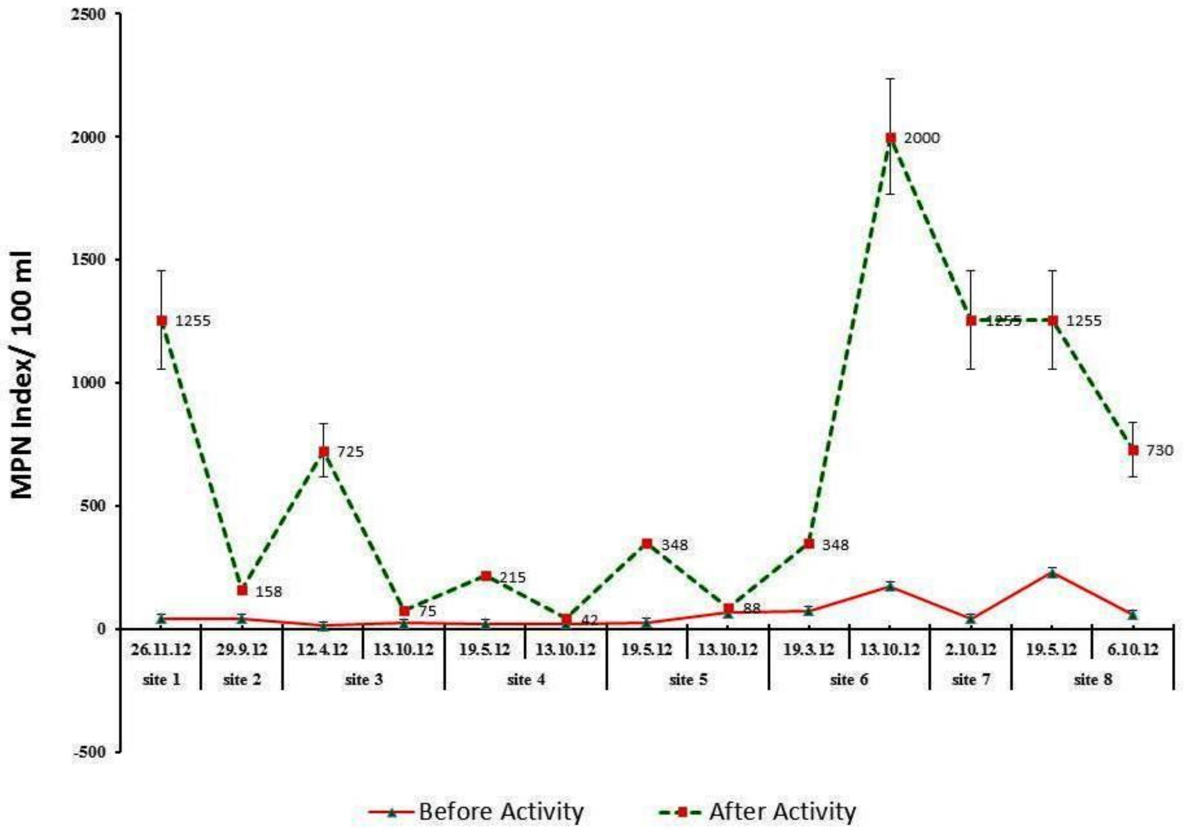

Figure 1: Standard Plate count of bacteria polpulation before and after the religious activities.

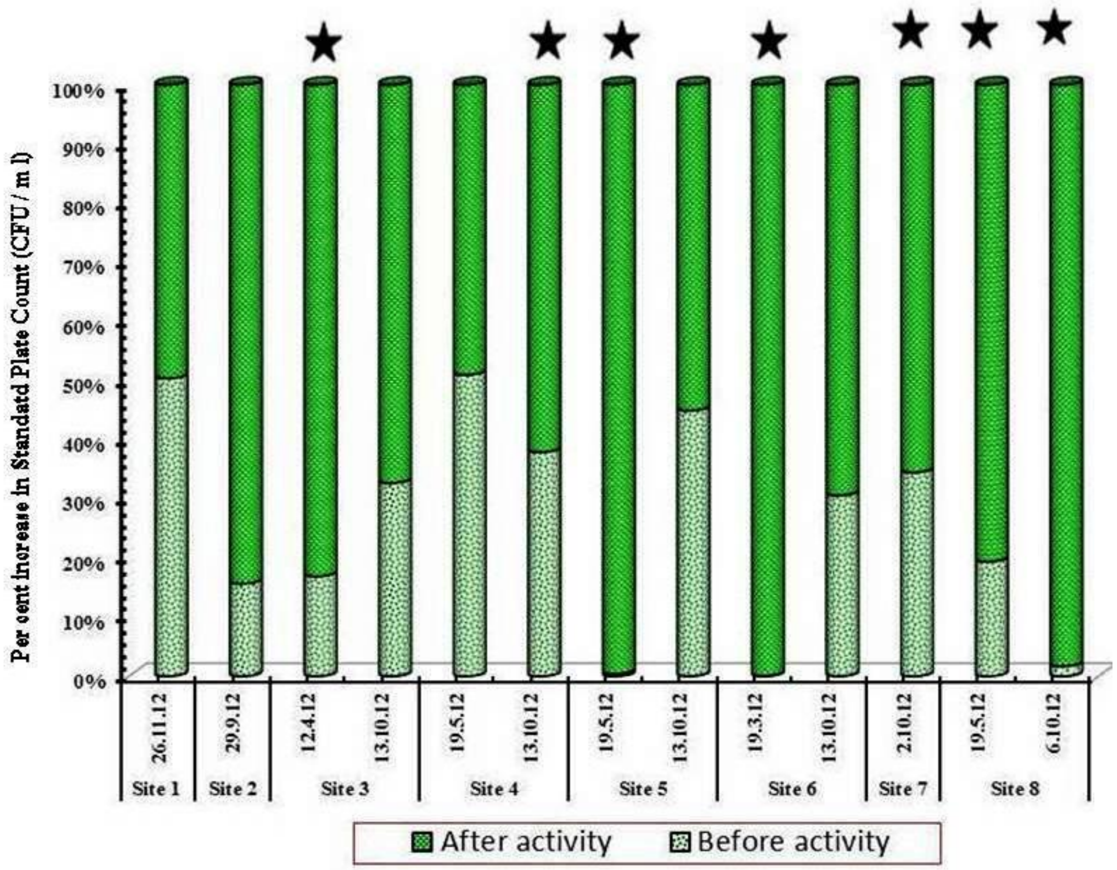

Figure 2: Per cent increase in total Coliform count before and after the religious activities ( ) ★ indicates significant differences (p<0.05).

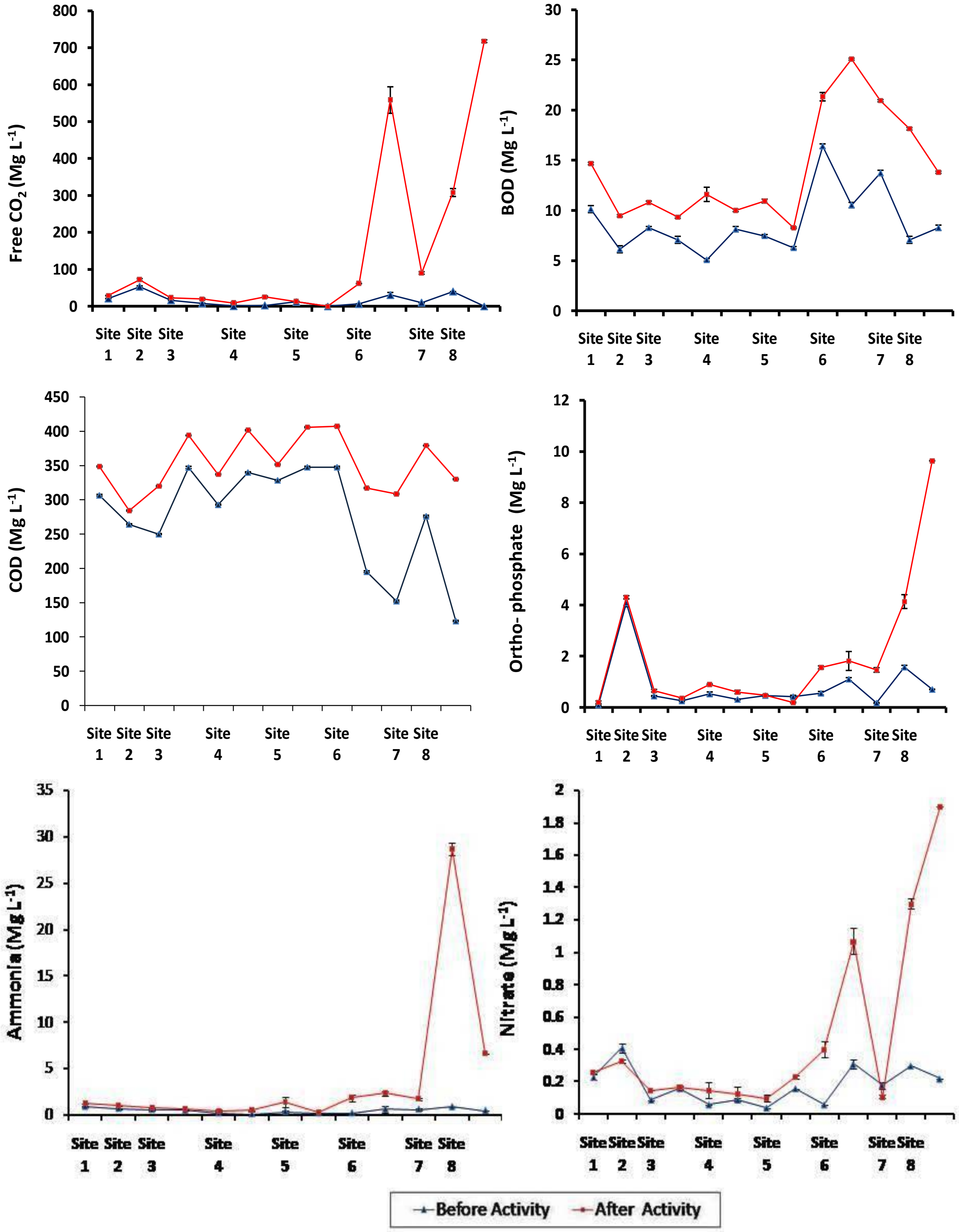

Figure 3: Variations in Free CO_2, Biochemical Oxygen Demand, Chemical Oxygen Demand, Ortho- phosphate, Ammonia and Nitrate before and after the religious activities at all the selected sites.

Various physico-chemical parameters viz. CO_2, NO_3^-, BOD, COD, o-PO_4^-, NH_4^+ were analyzed and an increase in the values of these parameters were recorded (Fig. 3) after the religious activity. Similar increases in the values of these parameters were also recorded by other scientists (Sinha *et al.*, 1991; Dhar *et al.*, 2004; Kulshrestha and Sharma, 2006) after such anthropogenic activities and the increasing concentration of o-phosphorus and nitrogen compounds in any aquatic system also responsible for eutrophication. Increase in the free CO_2, BOD, COD, o-PO_4^- after bathing may be due to dumping of biodegradable waste and contamination of water with human activities like addition of soaps, detergents etc. and their absorbance by aquatic flora. According to Buijs and Toader (2007) the level of CO_2 for bathing or recreation should be below 120 mg L^{-1} and the level of CO_2 was found below the permissible limits at all the sites (Table 4).

High value of the free carbon dioxide content is an indication of high degree of pollution, a fact also supported by Shah and Pandit (2012). The high organic matter and pollution load of pilgrims on the water bodies like bathing, adding ashes, flour, floral offerings, and urination may increase the nitrate and ammonia contents of water. Further a shift and disturbance in the optimum nitrification and denitrification processes in the waters may also be one of the reasons of the increased ammonia. The physico-chemical parameters were correlated with the bacteriological parameters via Pearson Coefficient of Correlation; a significant ($P < 0.01$) positive correlation of SPC of bacteria with the CO_2 ($r = 0.515$, $p < 0.01$), NO_3^- ($r = 0.728$, $p < 0.01$), PO_4^- ($r = 0.897$, $p < 0.01$) and with BOD ($r = 0.254$, $p < 0.05$) was observed. Most Probable Number of coliform count also showed a significant ($P < 0.01$) positive correlation with the parameters viz. CO_2 ($r = 0.631$, $p < 0.01$) and BOD ($r = 0.783$, $p < 0.01$) and with NH_4^+ ($r = 0.473$, $p < 0.05$), SPC ($r = 0.389$, $p < 0.05$). This positive correlation between BOD, COD, ammonia and free CO_2 with SPC and MPN clearly indicate that organic pollution after religious activities not only deteriorate the water quality in terms of increase in BOD, COD and ammonia but also increase the bacterial load (Standard Plate Count and MPN Index).

CONCLUSION

Overall in all the seasons water quality (in terms of physico-chemical and bacteriological aspects) of all the water tanks under study was found suitable and within safe limits for bathing purpose when compared with the water quality guidelines, but was not fit for drinking purpose. However, the quality of water was found to be deteriorated during the ritual occasions by mass bathing of millions of people and the religious activities performed by them and the values of physico-chemical bacteriological parameters were exceeding the limits as described by water quality standards for mass bathing. So, there is need to regularly change or mix the water after mass bathing. Educational campaigns involving recreational water managers or various stake holders at the local, national and international level need to be conducted to aware the public and also to identify periods when water quality is poor, issue advisory notices of warning the public of increased health risk, thereby protecting public health during recreational water use or ritual use. The conservation of the self-purification capacity of water bodies and waterways should also be an essential element of environmental protection programs as water undergoes self-purification and has potential for significant improvement in water quality if discharges are ameliorated. In order to ensure that water quality standards are not violated, there is need of regular monitoring and surveillance of water quality in terms of physico-chemical, biological and bacteriological parameters taking suitable remedial measures to control pollution and prevent the depletion of the quality of lentic waters.

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