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OBSERVATIONS ON *Salmonella typhi* IN KSHIPRA RIVER WITH RELATION TO ANTHROPOGENIC ACTIVITIES

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ABSTRACT

Salmonella typhi **is one of the leading cause of intestinal infections, typhoid and enteric fever. Contaminated food and water are one of the major vehicles for transmission of typhoidal *Salmonella*. Rivers play an important role in livelihood of individuals by providing potable water, cheap transportation, errigation, fisheries and electricity but these natural water bodies which serves as a blessing, can turn into disguise if it gets polluted as it becomes a potent source of contaminated water which is the major route for transmission of various pathogens . One year study was conducted from November 2013-October 2014 in river Kshipra which aimed to investigate occurrence, distribution abundance of *S.typhi* with relation to anthropogenic activities. Health impacts of *S.typhi* on the population which uses river water for different purposes like drinking, bathing and other domestic use are reported. Isolation of *S.typhi* was done by use of peptone water as a pre- enrichment medium, the obtained colonies were then transferred to Dicholorocitrate Agar for identification after which species were confirmed by applying different biochemical test. The study showed that most of the sites of the river were highly contaminated with *S.typhi* (2-158 x 103 CFU/100 ml.) that consistently increased WHO limits for potablity. Quantification of *S.typhi* revealed that its count was found to be higher in summer season and during onset of rain. However, a higher count was reported in bottom and the count of *S.typhi* showed marked variations with changes in different physicochemical parameters . The incidence of typhoid and enteric fever also showed similar seasonal pattern. The most impaired region were Ramghat, Mangalnath and Triveni study sites which receive different types of pollutants on regular basis via various sources. The mass holy dip by devotees significantly affect water quality by enhancing approximately ten fold increase in S.typhi count in study area. Public awareness, proper sanitary conditions and appropriate measures to control water pollution are required to provide lifeline to this sacred river.**

KEYWORDS : *Salmonella typhi*, bacteria, mass bath, anthropogenic activities, water pollution, Kshipra river, typhoid

Pollution of river water with deleterious microbes including bacteria, viruses, parasites and fungi has been on steady increase from recent past. The major source of microbes in water is via faeces of humans and animals. Pathogens enter in the river from both point sources, non-point sources, or from both. The impact of river pollution on human heath depends on use of water and concentration of pathogens. The agents find their way into water via domestic waste, agricultural land posture during rainfall or by direct deposition of faecal matter with access to stream channels (Collins et al. 2005). They are transferred to humans by recreational activities, irrigation of crops, vegetables and drinking water. Thus, detailed microbial investigation and regular water quality monitoring programs need to be conducted on regular basis to generate base line data for the welfare of society. One such microbe used for the assessment of water quality is Salmonella which is an ubiquitous, enteric pathogen with a world wide distribution, occurring frequently in raw sewage. Soil and sediment are known to harbour Salmonella and sediment particles are believed to function as a micro niche enhancing Salmonella survival in lakes and rivers. Certain species of Salmonella like *S.typhi* are pathogenic and are responsible for causing typhoid and enteric fever. *S.typhi* is used as an major indicator for assessment of water pollution of the river.

S.typhi is a rod shaped, Gram Negative, non-spore forming motile bacterium which is basically a serovar of enterica species of Salmonella and its major routes of contamination include food and water which is a major concern for public health. Thus, drinking and domestic water needs proper treatment in order to reduce the risk of water born diseases. With this concern the present study was planned and executed on the banks of river Kshipra. The main objective of this study was to evaluate the occurrence and distribution of *S.typhi* in Kshipra river and its health impact on population residing near to the river. Such a study is important as it shall provide a framework for practical measures to guide local authorities for river water management and conservation the river.

MATERIALS AND METHODS

Study Area

River Kshipra originates from a hill of Vindhya range, one mile south of Kshipra village lying 12 km south-east of Indore city (M.P.). It flows approximately between latitude 22º49' and 23º50'N, longitude of 75º45' and 75º35'.

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River flows across Malwa plateau to join river Chambal which later joins Gangetic system. In the present study, five study sites with high anthropogenic activities were selected on the banks of river Kshipra, they include Kshipra village, Triveni, Ramghat, Mangalnath and Mahidpur.

Water Sampling

Sampling was carried out monthly from November 2013 to October 2014 for isolation of microorganisms. Bacterial samples were collected aseptically using 500 ml sterile bottles and were kept in ice bucket, they were then transported to the base laboratory within 24 hours.

Isolation and Identification of *S.typhi*

The isolation of *Salmonella* strains was carried out using standard culture techniques (APHA 2005). Buffered peptone water was used as a pre-enrichment medium prior to selective enrichment for resuscitation of cells that have been injured. 10 ml of water samples were added to 90 ml distilled water 50 ml of buffered peptone water and incubated at 37^0 C for 16-20 hrs. For selective enrichment, Selenite Cystine broth and Rappaport- Vassiliadis Soya (RVS) broth were used simultaneously. These two enrichment media (10 ml) were individually inoculated with 0.1 ml of pre-enrichment buffered peptone water culture and then incubated at 42^0 C for 24-48 hrs. Obtained colonies were transferred to Dichlorocitrate Agar (DCA) for identification. Confirmation of species was done by applying various biochemical tests. *Salmonella typhi* is specifically identified by transferring appeared colonies on DCA media.

Analysis of Physicochemical Parameters

Sampling and analysis of various physicochemical parameters were done by using standard methods given in APHA (2005).

Water Born Diseases

Data on water born diseases was obtained by survey from major hospitals of Ujjain and Dewas city. A structural interview involving about 500 households randomly selected from all the four quarters in the city of Ujjain and Dewas. Respondents were required to furnish information on their sources of water for domestic use and the occurrence of water born diseases in their respective families.

RESULTS AND DISCUSSION

River Kshipra was mapped for detecting the presence of *S.typhi*. The results obtained indicate wide variations in the abundance, distribution and occurrence of *S.typhi* in Kshipra river system. During sampling period of one year 73.3% occurrence of *S.typhi* was registered in all the collected samples. The evaluated samples showed lower counts of *S.typhi* at Kshipra village (2 x 10^3 CFU/100 ml at surface and 5 x 10^3 CFU/100 ml at bottom) and Mahidpur (21 x 10^3 CFU/100 ml at surface and 30 x 10^3 CFU/100 ml at bottom). Comparatively, higher counts were reported at Ramghat (121 x 10^3 CFU/100 ml at surface and 158 x 10^3 CFU/100 ml at bottom), Triveni (73 x 10^3 CFU/100 ml at surface and 91 x 10^3 CFU/100 ml at bottom) and Mangalnath (96 x 10^3 CFU/100 ml at surface and 126 x 10^3 CFU/100 ml at bottom).

In Kshipra river system the overall count of *S.typhi* varied in different seasons throughout the year. At Kshipra village study site *S.typhi* was found to be absent in winter and monsoon seasons respectively. However, 1-3x10^3 CFU/100 ml colonies were obtained in the months of May and June. At Triveni study site 1-4x10^3 CFU/100 ml in winter, 6-10x10^3 CFU/100 ml in monsoon and 11-16x10^3 CFU/100 ml in summer were reported. At Ramghat study site 2-9x10^3 CFU/100 ml in winter, 9-16x10^3 CFU/100 ml in monsoon and 8-23x10^3 CFU/100 ml in summer were obtained. A count of 1-7x10^3 CFU/100 ml in winter, 7-15x10^3 CFU/100 ml in monsoon and 7-20x10^3 CFU/100 ml in summer were registered at Mangalnath study site. At Mahidpur study site *S.typhi* was found to be absent in winter, 2-4x10^3 CFU/100 ml were observed in monsoon and 5-7x10^3 CFU/100 ml were reported in summer (Figure1 and 2).

The mass holy dip by devotees significantly affect water and sediment quality by enhancing the *S.typhi* count in the study area. Three major mass baths falling on the occasion of Shanivari Amavasya (1st day of full moon falling on Saturday) covering all three seasons were studied. Samples were taken before, during and after mass baths continuously for ten days. A sharp increase of about ten fold in *S.typhi* count was registered in March (6x10^3 CFU/100 ml before dip to 65x10^3 CFU/100 ml after dip), (10x10^3

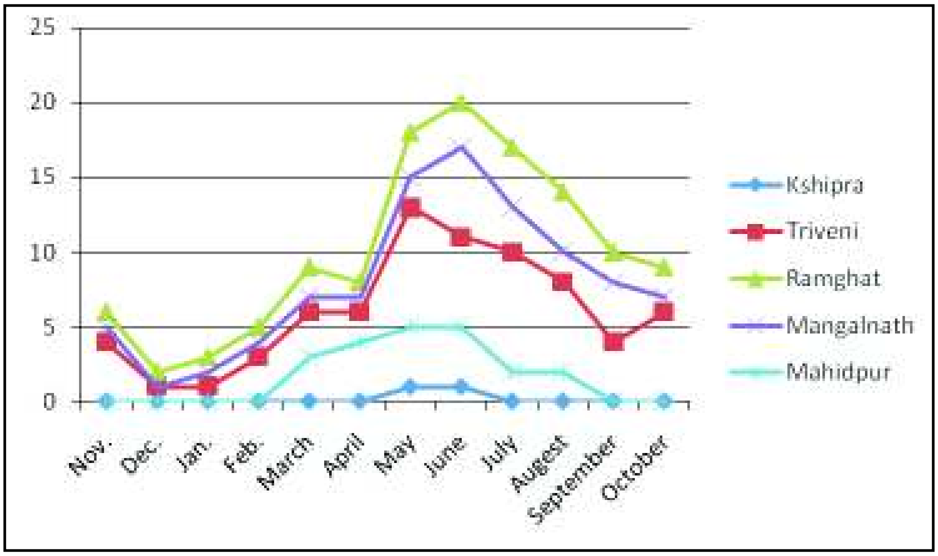

Figure 1 : Monthly Variations on *S.typhi* Count (Count x10^3 CFU/100ml) at Different Study Sites of Kshipra River (Surface Water).

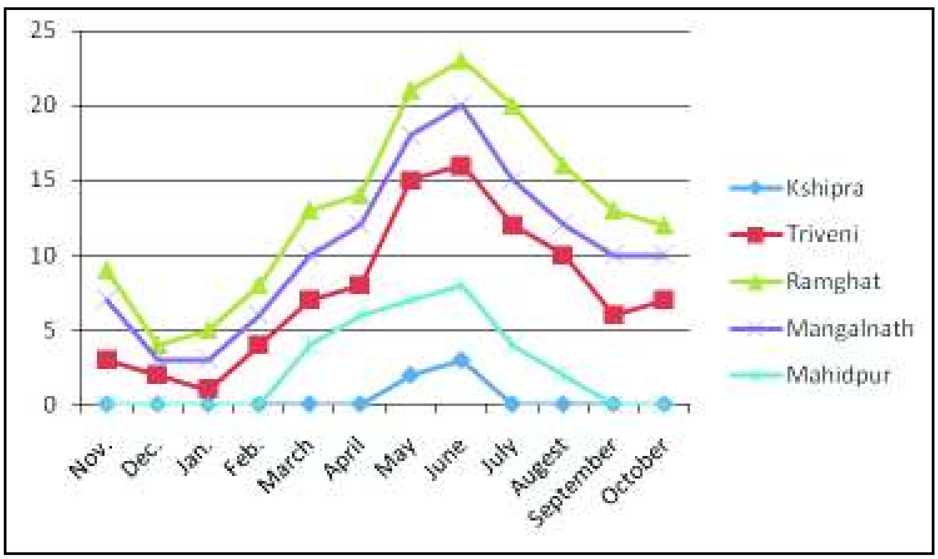

Figure 2 : Monthly Variations on *S.typhi* Count (Count x10^3 CFU/100ml) at Different Study Sites of Kshipra River (Bottom Water)

CFU/100 ml before dip to 89x10^3 CFU/100 ml after dip) in July and (4x10^3 CFU/100 ml before dip to 52x10^3 CFU/100 ml after dip) in November (Table 1).

Household interview is conducted in Ujjain and Dewas city showed that in most of the households, inhabitants residing nearby river constantly suffered from Typhoid, Enteric fever and gastrointestinitis. About 23-25% cases of typhoid and 15-18% cases of Enteric fever were reported in people residing near the river throughout study period. However, the rate of incidences increased during summer and early monsoon season when occurrence of *S.typhi* was higher. More than 60% people living near to the river use the river water for drinking, bathing and other domestic use.

In the present study *S. typhi* showed about 73.3% of *S.typhi* positive samples between period of November 2013-October 2014. The site wise distribution of *S.typhi* shows higher count at Ramghat followed by Mangalnath, Triveni, Mahidpur and Kshipra village. The pattern of spatial distribution is found to be in accordance with

Table 1: Anthropogenic Activities Observed at Kshipra River

S.No.	Activities	Study sites				
		Kshipra village	Triveni	Ramghat	Mangalnath	Mahidpur
1.	Mass bathing	-	+	+	-	-
2.	Flower dumping	-	+	+	+	-
3.	Coconut dumping	-	+	+	+	-
4.	Other rituals	-	+	+	+	-
5.	Oil leakage	-	+	-	-	-
6.	Ashes of dead bodies	-	+	+	-	-
7.	Presence of crimnetorium	+	+	-	-	-
8.	Domestic waste water disposal	+	-	+	+	-
9.	Tributary with industrial and domestic waste (Khan river)	-	+	-	-	-
10.	Agricultural runoff	+	+	-	-	+
11.	Boating	-	-	+	-	-

anthropogenic activities and pollution status of these sites. *S.typhi* was detected by various researchers in India and around the world. Tambekar et al.(2014) reported *S.typhi* from Wardha, Wainganga and Penganga rivers India. Diwivedi et al. (2014) recorded the presence of *S.typhi* in river Khan which is a tributary of Kshipra river. Rani et al. (2014) registered the presence of *S.typhi* in Ganga river India. Musyoki et al.(2013) reported $7.6x10^1$ colonies of *S.typhi* from Athi river before entering Narobi river and $2.1x10^2$ colonies of *S.typhi* after entering Narobi river Kenya. Abass (2008) reported 3.6% *S.typhi* from Euphrates river water Iraq. However in the present study $2\text{-}158x10^3$ CFU/100 ml were found indicating a higher pollution load of river Kshipra.

It is important to analyze the survival and reservoirs of *S.typhi* in environment to stop or lower down the spread of pathogens in the environment. High levels of *S.typhi* were observed in bottom water and sediments of river Ganga and its tributary Yamuna at Allahabad (Rani et al. 2014). These observations are well supported by the observations of the present study in which a higher count of *S.typhi* is registered in the bottom of the river. A colony count of $2\text{-}121x10^3$ CFU/100 ml were observed in surface water and colonies of *S.typhi* ranging between $5\text{-}158x10^3$ CFU/100ml were reported in the bottom water of the Kshipra river system. Higher count in bottom water may be attributed to sedimentation, absorption and possibly extended survival of pathogens in aquatic sediments. The presence of a higher number of organic substance, phytoplankton, phosphate and nitrate get absorbed to sand grains, clays etc. is a factor advantageous for proliferation and survival of these bacteria in bottom sediments of the river.

The count of *S.typhi* was found to vary with in different seasons. Authur et al. (2015) reported *S.typhi* count and cases of Typhoid to increase with rainfall in Ghana. However in the present study reports maximum count of *S.typhi* in summer ($1\text{-}23x10^3$ CFU/100 ml) and minimum in winter ($0\text{-}9x10^3$ CFU/100 ml). Similar findings were reported by Awah et al. (2013) who found maximum Salmonella counts during summer and minimum counts during winter seasons respectively. Since, India is a tropical country where large variations in environmental factors like temperature and humidity are observed which are responsible for marked variations in Salmonella count throughout the year. In summer season direct deposition of faecal matter is there in the river which is compounded by minimum dilution due to reduced river flow. However, during rainy season regular rainfall flushes faecal matter from land as they are deposited with increased volume of water in river channels, resulting in maximum dilution and lower counts.

In the present study seasonal variation of atmospheric temperature ranged between $15.8^0C\text{-}38.2^0C$, water temperature between 15.3^{0}C-30.8^{0}C, pH within 7.9 - 8.7 and dissolved oxygen was found between a range of 4.1-7.8 mg/litre. Biological oxygen demand was found within a range 8-37.6 mg/litre, Chemical oxygen demand ranged between 25.6-153.9 mg/litre. Lower values of dissolved oxygen, higher values of biological oxygen demand and chemical oxygen demand were observed at Ramghat and Mangalnath study sites. This finding is well supported by a high microbial count at these sites, which indicates pollution load in the water body. However, high dissolved oxygen, lower biological oxygen demand and chemical oxygen demand was found at Kshipra village indicating comparatively less polluted with respect to other sites. As far as seasonal trends are concerned low dissolved oxygen were observed in summer because of increased temperature which decreases gas solubility of water (Bhattaraj, 2008). High biological oxygen demand and chemical oxygen demand values were observed in summer which is due to low water level, enrichment of nutrients and organic matters which supports microbial growth. During summer season, more mass baths and worship rituals are performed in the river due to which an increased organic load and microbial count were observed. The occurrence of *S.typhi* in river Kshipra is positively correlated with temperature, pH, BOD, COD and turbidity and a negative correlation with dissolved oxygen and transparency is observed.

Environmental status of Kshipra river is influenced by various factors like urbanisation, industrialisation, accumulation of effluents, intense agricultural operations and faecal contamination. The count of *S.typhi* is known to increase with these activities. So, a higher count of *S.typhi* is reported at different studied sites of Kshipra river. Lack of proper sanitation facilities in urban cities has been citied as the main cause of high bacterial pathogens in rivers transversing major cities in the world. Million litters of untreated sewage are discharged in the river Kshipra which carries high bacterial load. The occurrence of *S.typhi* is correlated with proximity of water contamination by sewage discharge and anthropogenic activities. About 12-15 lake population of Dewas and Ujjain district depend on water of river Kshipra for irrigation, domestic and drinking purpose. Crops irrigated by this water are consumed by inhabitants of these and neighbouring cities which gives rise to outbreak of water born diseases. High values of *S.typhi* are obtained from Ramghat, followed by Mangalnath and Triveni. These increased values are evident because of high anthropogenic activities and human interventions viewed at these sites (Table1). Ramghat is the main bathing centre of Ujjain city where many festive mass baths, accompanied by various worship rituals like flower dumping, ashes dumping etc. are performed. Triveni also witnesses mass baths on different occasions which are participated by pilgrims across the world. The site is demarked by presence of a Shani temple and local crimnetorium. At this site oil leakage is observed as a result of which oil directly enters into the river increasing pollution load of the river. This study site is also the meeting point of river Khan which receives about 1500 m^3 of textile effluents having all poisonous textile dyes with oil and grease resulting in higher BOD and COD. At Sawer about 4000 m^3 sludge is added into Khan river. Water from here is drawn for irrigation of about 600 acres for growing vegetables (Rao et al.1992). Similarly at Mangalnath also different worship rituals are observed which increase nutrient concentration, organic pollution and microbial load of the river. Presence of brick making activity is observed about 100 brick kilns have damaged the flood plains of the river. These pollutants enter the river and contribute to the increased pollution at Ramghat and Mangalnath sites. Kshipra village is near to the origin point of Kshipra river because of which a lower count of *S.typhi* is observed over here. However, this site is marked by comparatively less anthropogenic activities. Similarly, lower counts of *S.typhi* are observed at downstream of Mahidpur which are attributed to self-purification capacity of the river and also because of the distance of 150 km from the origin point which reduces the number and concentration of bacteria.

Mass bath is an old ritual in India and is one of the main cause of water contamination of our holy rivers. They leads to increased nutrient concentration, organic and inorganic substances in the water body along with different pathogenic microorganisms, which remains constant for a longer period of time (Table 2). Mass bath serve as a hazard for water quality because when million of pilgrims enter in Kshipra or any other river ecosystem the settled silt, clay

Table 2 : Occurrence of *S.typhi* During Mass Bath (Shanivari Amavasya i.e. First Day of Full Moon Falling on Saturday) at Triveni Study Site of Kshipra River

1.	Mass Bath date 1/3/2014 →	Pre Dip	During Dip	After Dip								
		28 Feb.	1 Mar.	2 Mar.	3 Mar.	4 Mar.	5 Mar.	6 Mar.	7 Mar.	8 Mar.	9 Mar.	10 Mar.
	S.typhi count X 10^3 CFU/100 *ml*	6	45	60	63	65	61	58	49	33	22	13
2.	Mass Bath date 26/7/2014 →	Pre Dip	During Dip	After Dip								
		25 Jul.	26 Jul.	27 Jul.	28 Jul.	29 Jul.	30 Jul.	31 Jul.	1 Aug.	2 Aug.	3 Aug.	4 Aug.
	S.typhi count X 10^3 CFU/100 *ml*	10	70	86	89	87	85	75	63	48	39	20
3.	Mass Bath date 21/11/2014 →	Pre Dip	During Dip	After Dip								
		21 Nov.	22 Nov.	23 Nov.	24 Nov.	25 Nov.	26 Nov.	27 Nov.	28 Nov.	29 Nov.	30 Nov.	1 Dec.
	S.typhi count X 10^3 CFU/100 *ml*	4	40	49	51	52	44	31	28	18	14	12

and sand mixes with the surface water which enhances total hardness, calcium, chloride, total alkalinity, and other metals present in the soil of the river. Due to this intense mixing of surface and bottom water only surface water samples were collected during mass bath. Increased values of all physicochemical and microbiological parameters were observed during and after mass baths which returned to normal position after a long period due to self purification capacity of river (Bhasin et al.2015). Mass bath is ruining ecology of river Kshipra from a very long time. The Ujjain city hosts Mahakumbh (mass bath) which greatly influences river ecosystem especially at Ramghat. The massive mass bath had its influence on the water quality of the particular site for about an year. The accumulation of soap films, hair, dead skin, body oil, dirt, faeces, throwing floral offerings, discarded cloths, spitting and answering nature's call in the river by devotees in the river create ideal conditions for bacterial survival and multiplication. Similar trends were reported earlier by Tyagi et al. (2013) in river Ganga. An important fact pictured during the study period was that an increased count of typhoid and enteric fever (3-5%) was reported from major hospitals of the city. The reported cases include people residing near the river and pilgrims who took bath in the river. However, many cases of typhoid and enteric fever remain unnoticed as devotees went back to their towns and could not be contacted.

Typhoid and Enteric fevers are systemic diseases caused by human host adapted *S.typhi*. Both epidemic and endemic incidences of typhoid is a major health issue resulting in high economic burden, mortality especially in a developing country like India. In the present study 23-25% cases of typhoid and 15-18% cases of enteric fever were registered throughout study period, which were found to increase by 3-5% during mass baths particularly for typhoid cases. Higher cases were reported during summer season due to increased count of *S.typhi*. Similarly, an epidemiological survey conducted during mass bath in Ganga river reported total 5368 cases of water borne infections due to deteriorated microbiological quality of Ganga water (Tyagi et al. 2013). During Summer 2002 within a seven week period 5963 cases of Typhoid fever were reported in Bharatpur Nepal (Lewis et al. 2005). The outbreak was found to be associated with high *S.typhi* counts in municipal water. In Africa high count of *S.typhi* in drinking water was found to be associated with high cases of typhoid fever more than 100 cases per 10,000 individuals were registered (Crump et al.2004). According to CDC data in USA from 1971-2000 an increase of 6% in typhoid cases were observed which was less than that in developing countries (Craun et al.2004). However in England Salmonella was never associated to water borne outbreak in England and Wales from 1992 to 2003 (Smith et al. 2006) indicating that Salmonella count and infection are greater in developing countries than developed countries.

In developing countries, the main source of river pollution is mainly via faecal contamination, discharge of untreated waste and sewage in the water body, lack of proper sanitation facilities and agricultural run off. However, in such countries lack of water supply, self-sustaining decentralised approaches including point of chemical and solar disinfection, safe water storage and behaviour changes are indicated as reliable options to directly target most affected population and reduce water-born disease burden through improved drinking water quality. In developed countries, industrial effluents, agricultural runoff and mixing of pesticides and fertilizers with the river or tap water contributes as a major source of water contamination. In such industrialised countries, the success of applied control strategies is confirmed by small number of water- born outbreak caused by various water born microbes Nevertheless, outbreaks caused by microbial contamination of drinking water still result in substantial human and economic cost in these countries. In a resource constrained country like India, surface water is used for drinking, bathing, recreational and holy activities. However, factors like sewage and waste discharge, industrial effluents, agricultural runoff contribute to increase the level of pollution in Indian river, but another factor which is a very important reason for pollution of Indian river system is the occurrence of religious festivals conducted on the banks of major Indian holy rivers like Ganga, Yamuna, Godavari and Kshipra. The river water gets flooded with many worship rituals and this water if used without proper treatment can lead to various health hazards. River Kshipra hosts the Mahakumbh mela which is a religious festival organized in every twelve years attracting millions of tourists and devotees from all around the world to take bath in this sacred river, this gives rise depletion water quality. River Khipra is known to originate from lap of Lord Bhrama hence, it enjoys status of Goddess in Hindu mythology. Hence, religious activities like dumping of body ashes, flowers, coconut shells and statues is evident at the banks of this river. These activities certify that in holy rivers of India mode and nature of pollution is different from water bodies across the world.

CONCLUSION

The high count of *S.typhi* obtained in the study indicate that river Kshipra is receiving faecal, domestic waste, organic and sewage pollution on regular bases. The major source of pollution in Kshipra river are the performance of extensive worship rituals and anthropogenic activities like dumping of flowers body ashes coconut shells etc. as a result of which sites with high human interventions like Ramghat, Triveni and Mangalnath show higher counts of *S.typhi*. Higher count of *S.typhi* were registered in summer due to reduction in water volume and a higher count was reported in bottom water due to absorption at the bottom. Similarly, an increase in cases of typhoid and enteric fever during summer seasons were observed. Regular occurrence of mass baths on different festive occasions has destroyed the rivers ecology to a great extent leading to an increased *S.typhi* count and depletion of water quality. The aim of this study is not to create a horror image of mass baths nor does the study focuses at opposing various worship rituals, but the study strongly recommends use of certain eco friendly methods like use of degradable material for making statues of Lord Ganesha so it does not disturb the water quality of the river to a lesser extent. The water of Kshipra river is unfit for consumption without proper treatment process. This in turn can pose many problems for people residing in close by vicinity and can cause many water born diseases. Regular water monitoring, and public awareness are required to improve the present sanario. Disposal of coconut, flowers, industrial and city sewage should be totally prohibited in this holy river. This reaffirms the need for regular monitoring of microbial parameters of the water body, in order to minimize the risk of infection to exposed person. A proper action like Ganga Action Plan needs to be initiated by State and Central government for cleaning and conservation of this sacred river. A level of understanding is required by devotes and pilgrims as they should coordinate with government and local authorities for conservation of this holy river.

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