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Phul Kunwar Singh Rana
Assistant Professor of Zoology, V.R.D. Govt. College, Marwahi G.P.M. Chhattisgarh, India

Corresponding Author:
Phul Kunwar Singh Rana
Assistant Professor of Zoology, V.R.D. Govt. College, Marwahi G.P.M. Chhattisgarh, India

Occurrence and distribution of pollution indicator bacteria in shellfish harvesting waters of Chandan ghat, Narmada River, Dindori

Phul Kunwar Singh Rana

Abstract
Present study focuses on the seasonal variation in coliforms and the total bacterial load in bank waters of the Chandan ghat, Narmada river, Dindori district from October 2019 to September 2020. Temperatures and salts of water were found in the standard range and there was a significant difference in the number of bacteria and the total number of Vibrios found in the study areas. From a seasonal variability study, it was found that coliforms and the total number of bacteria were significantly higher during the monsoon and after the post-monsoon.

Keywords: Bacteria, bank, fecal coliform, total coliforms

Introduction
Banks are inhabited by several species of bivalve molluscs including the commercially important edible bivalves such as clams (*Meretrix* spp), oysters (*Crassostrea* spp) and mussels (*Perna viridis*), that support the livelihood of local fishermen. These ecosystems, which frequently support the commercial production of bivalves within intertidal zones are exposed to contamination with human sewage and animal wastes, and thus pose risks to human health resulting from the consumption of contaminated bivalves (Carlos and Campos, 2007) [1]. The quality of bivalve harvesting as well as growing water is determined by the total bacterial load, fecal coliform bacteria (FC) and physico chemical parameters (Knap *et al.* 2002) [2]. The coliform bacteria are considered as an indicator of sewage associated pathogen (USA, 1999) [3] and higher the levels of indicator bacteria higher will be the level of the fecal contamination and greater will be the risk of the diseases (Scott *et al.* 2003 and Pipes, 1981) [4, 5]. The growth and survival of fecal indicator bacteria are affected by environmental factors such as water temperature, salinity and rainfall (Namdeo and Singh, 2021) [6]. Fecal coliforms are commonly used as indicator of sanitary quality that helps to assess possible presence of fecal and pathogenic organisms and thereby ultimately evaluating the quality of environment. Thus study was focused on understanding the seasonal differences in all microbial groups looked for, so as to provide an annual cycle of microbial load in major banks along the Narmada river of Amarkantak.

Material and methods
Narmada river is an important river of India. It is also known as a life line of Madhya Pradesh and its origin from Amarkantak of Anuppur district. Amarkanatak is second largest plateau of M.P. It has height of 1051 meter from sea level and flows through M.P. and Gujarat states. It falls into bay of Arabian sea (Khambhat) Bharuch. The length of this river is 1312 km. out of which about 1077 km. falls in M.P. Chandan ghat is one of the important ghats of river Narmada. Local inhabitants go to take holy dip in this ghat.
The water samples were collected from Chandan ghat, Narmada river Dindori district in Madhya Pradesh once in a month for the period of one year from October 2019 to September 2020; comprising of pre-monsoon (February - May), monsoon (June - September) and post-monsoon (October – January) seasons. The water sample was collected in presterilized plastic sampling bottles and the temperature of the water was measured using standard mercury filled centigrade thermometer at the sampling site itself. Samples were transported

to the laboratory and subjected to bacteriological examination within 4 hours of collection for total bacteria, total Vibrio and coliform counts. The salinity of water was estimated by titrimetric method. (Strickalnd and Parsons, 1972) [7].

For bacteriological examination, one ml of appropriate diluted water sample was spread plated on Zobell Marine Agar for the total bacterial count (TBC) and Hichrome Vibrio Agar for the total Vibrio count (TVC) and incubated at 37°C for 24 hours. After the incubation period the total number of colonies was counted and counts were expressed in terms of Colony Forming Unit (CFU) per milliliter of the sample. For enumerating the total coliform (TC) and fecal coliforms (FC), five tubes Most Probable number (MPN) test was performed by using Lauryl Sulfate Tryptose Broth (LSTB) and *Eschericia coli* (EC) broth, respectively. For the total coliforms; 10ml, 1ml and 0.1 ml test samples were added to each set of 5 tubes and incubated at 37°C for 24 hours. A loop of culture from the positive MPN test tube was transferred to 5ml of EC broth tubes and incubated at 44.50C for 24 hours in the water bath for the enumeration of the fecal contamination. The positive results were indicated by the growth (turbidity) and the accumulation of the gas in Durham's tubes. The coliforms numbers were expressed as counts/100ml referring to Mac Cradys' table (1918) [8].

Results

The monthly samples were collected and subjected to microbiological investigations for one year from October 2019 to September 2020, comprising of pre monsoon, monsoon and post monsoon seasons. Average values were calculated based on the monthly sampling results for all the seasons. Salinity of the water during the study period ranged from 9.8 to 34.2 ppt in Site-I, 10.7 to 35.9ppt in Site-II, 13.1 to 33.8ppt in Site-III and 18.2 to 29.7 ppt in Site-IV at Chandan ghat, Narmada river. In all the sites, the average high salinity was observed in the pre-monsoon season and lowest was during the monsoon season (Table 1).

Water temperature did not show much variation during the study period. The values recorded ranged from 23 to 25.4°C in Site-I and 24.2 to 27.2°C Site-II, 23.6 to 27.2°C Site-III and 23.4 to 25.8°C in Site-IV Chandan ghat, Narmada water. High and low temperature conditions were observed respectively in all study sites during the pre-monsoon season and lowest was during the monsoon seasons (Table 1). The mean $\log_{10}$ MPN count of coliforms recorded for the study sites are given in Table 2. The TC and FC in the bank water from the sampling sites are presented in Fig. 1 and Fig. 2. Mean $\log_{10}$ MPN counts of coliforms were found to be 2.9 for TC and 3.2 for FC /100ml from all the study sites. Prevalence was significantly higher ($p<0.05$) during the monsoon season with the count of 3.201 compared to pre-monsoon and post-monsoon season at Chandan ghat, Narmada river.

Table 1: Seasonal variations in the environmental parameters of water samples from the study sites.

Study sites	Premonsoon		Monsoon		Postmonsoon	
	Temp.(°C)	Salinity (ppt)	Temp.(°C)	Salinity (ppt)	Temp.(°C)	Salinity (ppt)
I	25	34.2	23	9.8	24.1	25.4
II	27.2	35.9	24.2	10.7	24.3	22.1
III	27.2	33.8	23.6	13.1	24.8	29.6
IV	25.8	29.7	23.5	18.2	23.4	28.6

Table 2: Seasonal trends (mean± standard error) in $\log_{10}$ MPN coliform counts of water from the study site.

Study sites	Pre-monsoon		Monsoon		Post-monsoon	
	TC	FC	TC	FC	TC	FC
I	3.201	2.685±0.6	3.201	2.221±0.08	3.201	3.201
II	2.665±0.6	3.201	3.201	3.203±0.9	2.928±0.2	2.928±0.2
III	2.221±0.4	2.221±.05	3.201	3.201	2.416±0.3	2.427±0.5
IV	3.201	2.21±0.8	3.201	3.201	2.266±0.3	2.267±0.4

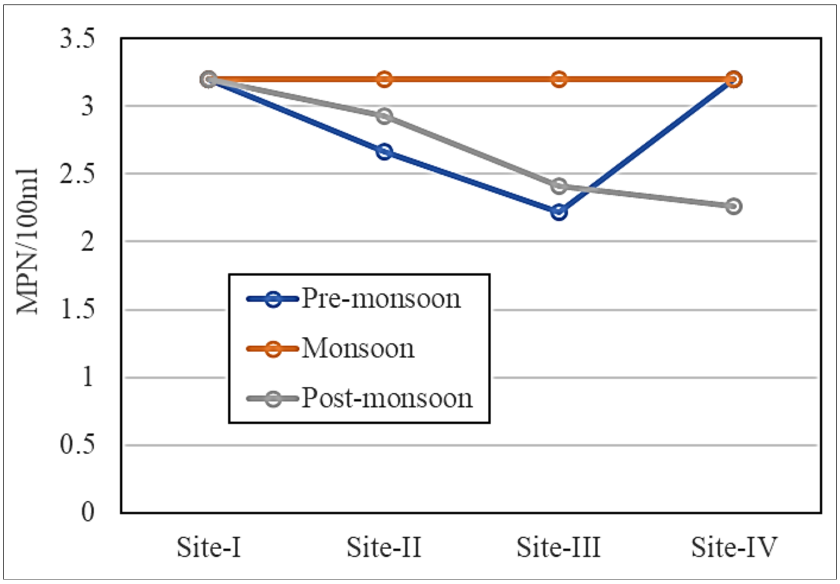

Fig 1: Total coliform of water collected from shellfish growing

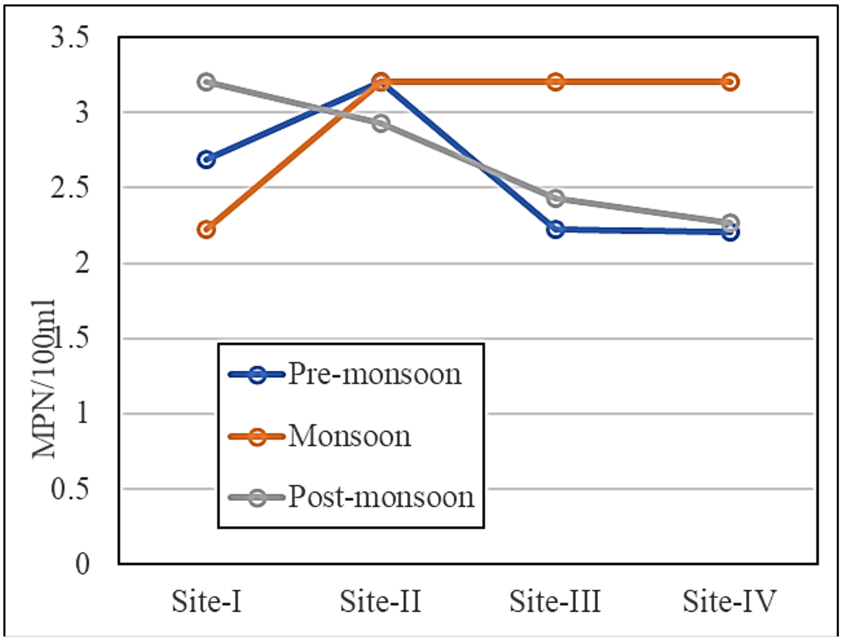

Fig 2: Fecal Coliform of water collected from shellfish growing

The limits of fecal coliform (FC) are set below geometric mean (GM) of 88 MPN/100ml with lesser than 10% of samples exceeding 260 MPN/100ml for a five tube MPN. GM of FC of the annual counts were 1066 MPN/100ml (80% of the sample) at site I, 885MPN/100ml (36% of the sample) at site II, 361MPN/100ml (54% of the sample) at site III and 417MPN/100ml (72% of the sample) at site IV waters (table 3). These values exceed the FC levels of 88 MPN GM/100ml water established by NSSP guidelines.

Table 3: Distribution of fecal coliforms (FC) (GM, MPN/100ml) in shellfish harvesting waters from different sites based on the limits imposed by NSSP

Sites	Seasons	FC(GM)	90th percentile	%>260MPN/100ml
I	Pre monsoon	497	1600	66
	Monsoon	1600	1600	100
	Post monsoon	1330	1600	100
	Annual	1060	1600	80
II	Pre monsoon	885	1600	75
	Monsoon	1600	1600	100
	Post monsoon	402	1328	33
	Annual	885	1600	36
III	Pre monsoon	167	695	25
	Monsoon	1600	1600	100
	Post monsoon	139	328	33
	Annual	361	1600	54
IV	Pre monsoon	293	1600	25
	Monsoon	1600	1600	100
	Post monsoon	111	296	33
	Annual	417	1600	72
US FDA Classification	Geometric mean	90th percentile	Shellfish treatment required	Criteria
Approved	MPN<14/100ml	MPN<43/100ml	None	Acceptable water quality; No significant pollution sources
Restricted	MPN<88/100ml	MPN<260/100ml	Depuration or relaying	Evidence of marginal pollution
Prohibited		No harvest allowed		Evidence of gross pollution

In site I waters, the geometric mean of FC during premonsoon season was 497 MPN/100ml, during monsoon 1600 MPN/100ml and during post monsoon 1330 MPN/100ml. These counts were above 14MPN/100ml and the 90th percentile was higher than 43MPN/100ml. In site II GM of FC was found to be 885 MPN/100ml in pre monsoon, 1600 MPN/100ml in monsoon and 402 MPN/100 ml in post monsoon seasons and these have exceeded 90th percentile imposed by NSSP guidelines. In site III, during pre-monsoon (167/MPN/100ml), post monsoon (139MPN/100ml) and monsoon (1600 MPN) seasons had values higher than 90th percentile imposed by the standards. In site IV, pre monsoon (293 MPN/100ml), monsoon (1600MPN/100ml) and post monsoon (111MPN/100ml) period showed higher than 43MPN/100ml, which is 90th percentile, imposed by the NSSP guidelines. The GM FC MPN recorded higher count during the monsoon season in all the study sites. FC levels in the 90% (site I, II), 81% (site III) and 72% (site IV) of samples were above 88MPN/

100ml of GM, which can be considered as "restricted area" according to the classification.

Total heterotrophic bacteria recorded from the study sites are presented in Table 4 and Fig 3 and Fig 4. The mean log_{10} of the colony forming units counted were taken. Seasonal trends indicated the significance differences ($p<0.05$) with higher bacterial count in monsoon (site I: 1.14± 0.06; site II: 0.12±0.13 and site IV: 1.28+0.06). In site III higher bacterial count was observed during the Pre monsoon season (1.12± 0.04) than monsoon and post monsoon season Table 4.

Table 4: Seasonal trends (mean+ standard error) in log_{10} count of TBC and TVC in CFU/ml of water from the study sites.

Seasons	Site – I		Site – II		Site – III		Site - IV	
	TBC	TVC	TBC	TVC	TBC	TVC	TBC	TVC
Pre monsoon	1.00±0.02	0.0	0.96±0.03	0.35±0.35	1.12±0.04	0.62±0.11	1.13±0.01	0.14±0.11
Monsoon	1.14±0.06	0.0	1.12±0.13	0.0	0.85±0.05	0.0	1.28±0.06	0.87±0.6
Post monsoon	1.05±1.03	0.45±0.33	1.06±0.24	0.22±0.51	0.81±014	0.05±0.4	0.96±0.91	0.21±0.21

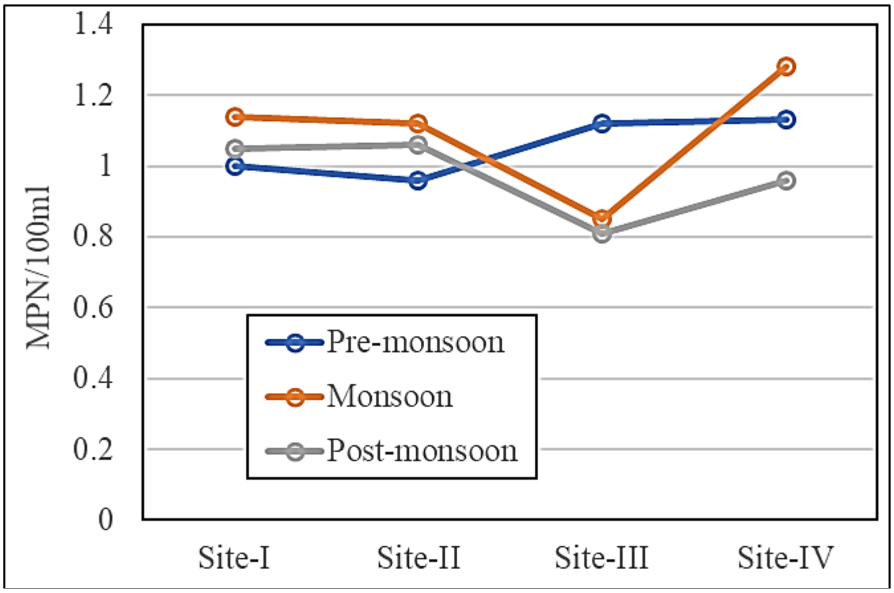

Fig 3: Total bacterial count (TBC) of water collected

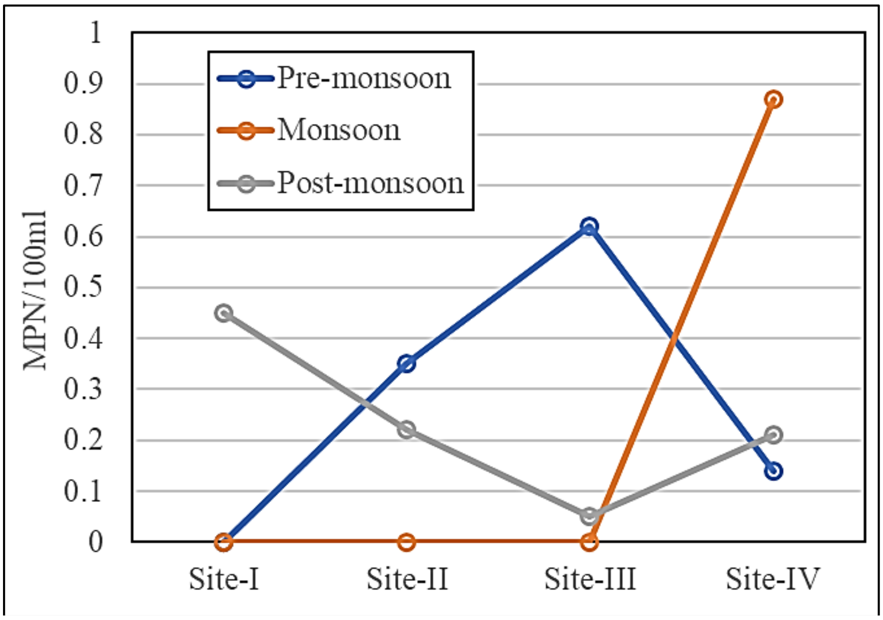

Fig 4: Total Vibrio count (TVC) of water collected

TVC from the samples are presented in Table 4 and Fig 4. Seasonal trends indicated the significance differences ($p<0.05$) with higher counts during Post monsoon season in site I (0.45±0.33), pre-monsoon season in site II and site III (0.35±0.35, 0.62±0.111, respectively) and during monsoon (0.87± 0.6) season in site IV. There was no incidence of Vibrio during monsoon season in site I, site II and site III.

Discussion

In recent years, the estuarine environments are subjected to increased anthropogenic activities leading to discharge of raw or partially treated domestic sewage thereby contaminating the estuarine environment with human pathogenic microorganism.

Pathogen indicator organisms such as coliforms that are associated with the sewage are chosen because they are easy to isolate and they help to identify the source of contamination and microbial quality of water (Geonha, and Jin, 2010, Fujioka, 2002, Tyagi, *et al.* 2006) [9-11]. Presence of microorganisms of fecal origin is the clear indication that contamination has occurred. Sewage contamination would lead to increase in number of coliforms in natural water body and also in the parts of animal living in these water bodies (Namdeo and Singh, 2021b) [12]. Numbers of fecal coliform bacteria are widely used as microbiological parameters indicating fecal pollution, while those of total coliforms that are of fecal but also of environmental origin can serve as a parameter to provide basic information on surface water quality (Namdeo and Singh, 2021b) [12]. Higher the incidence of indicator bacteria in waters, higher would be the chances for human pathogenic bacteria to be present. During monsoon months, the water body experiences the enhanced flow of water and the mixing up of the domestic sewage (WHO, 2001-02) [13]. In the present study the coliform count was found to be highest during monsoon season and similar pattern of highest coliform in monsoon season was reported in earlier studies from different parts of India (Bhagde, and Verma, 1982, Bharadwaj, 2005, Kumaraswamy, *et al.* 2009 and Namdeo 2021) [14-17] and TBC count was also found to be highest during monsoon season and the similar reports were observed in the other studies by several researchers (Ramaiah, *et al.* 2001, Mohandass, *et al.* 2003) [18, 19]. Increased pollution of water bodies generally found during monsoon due to consequence of heavy surface runoff also provides the inputs of pathogenic microorganisms into the water. In the present study bacterial load again increased during the post monsoon season with the reduced water flow, which was similar to the study by Ramaiah *et al.*, (2002) [18]. In tropical countries the seasonal cycle of the Vibrio is correlated with the rainy and dry seasons; the lowest numbers are found in rainy months, and the highest numbers are found in the dry season (Namdeo and Singh, 2001a) [6]. Our observations suggest a similar trend with higher count of Vibrio during premonsoon season from all the study stations. Onset of monsoon contributed to the decrease in salinity and temperature which was similar to the findings of Sharma (2015) [19], Gowda *et al.*, (2002) [20] and Sasikumar *et al.*, (2002) [21]; values observed for post monsoon, pre monsoon and monsoon seasons were found to be normal to the tropical estuary. Salinity value observed was closer to salinity of marine waters and the salinity fluctuation in study site is influenced by the tidal condition with high salinity during high tide and low salinity during low tide conditions. The temperature conditions were in ideal range for the growth of mesophilic bacteria.

Use of coliform group of bacteria as indicator organism for assessing the microbiological quality of water for drinking purpose is used as the standard procedure worldwide (Bergstein-Ben, *et al.* 1997) [22]. Among enteric bacteria *E. coli* is a common commensal gut flora of human and is discharged along with feces. Thus, its presence is used as an indicator of pathogenic microorganisms in natural and treated water (Hamilton, *et al.* 2005) [23] and use of indicator organism help to identify the source of contamination (Geonha and Jaeyoung, 2011) [24]. According to the coliforms limit imposed by National shellfish sanitation program (NSSP) (USFDA, 2013) [25], the water sample which gives geometric mean <14 MPN/100ml with 90th percentile of <43 MPN/100ml, it comes under the category of "Approved" and it does not require any shellfish treatment, if geometric mean <88 MPN/100ml with 90th percentile of <260 MPN/100ml, it comes under the category of "restricted", which shows the evidence of marginal pollution and thus suggested for the depuration or relaying of the harvested shellfishes. From our present study, 100% of the samples collected from all the stations during monsoon seasons were having MPN values >260/100ml (Table 4), which belonged to "restricted area" as imposed by NSSP guidelines. Thus, the water samples from all the study sites did not reach the sanitary standards.

Conclusion

Indicator bacteria such as total coliforms and fecal coliforms have been used as a model for the study of pathogenic microorganisms. The present study dealt with the seasonal variation and the distribution of the microbial count from the study sites of Anuppur and Dindori district, Madhya Pradesh, India. This study reveals the influence of anthropogenic activities at the coastal water in terms of incidence of feces associated indicator organisms.

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