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Full Length Research Paper

Assessment of bacterial population of River Barak and Its Tributaries, Assam, India

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The present work was undertaken with the objective to isolate bacteria from the aquatic fresh water ecosystem of Barak River and its tributaries and to assess the microbial contamination due to human activity. Water samples were collected from 14 different sites and analyzed for physicochemical and bacteriological parameters. On the basis of their morphological and physiological characteristics, a total of 23 bacterial isolates belonging to 8 genera were identified which include 26.09% *Pseudomonas*, 21.74% *Staphylococcus*, 13.04% *Escherichia coli*, 13.04% *Sarcina*, 8.69% *Enterobacter*, 8.69% *Shigella,* 4.35% *Serretia*, and 4.35% *Klebsiella*. The water of river Barak and its tributaries had been found to be largely polluted due to domestic and agricultural waste disposal, discharge from sewage and industrial effluents, animal defecation and waddling, bathing and washing, fishing and swimming etc. Mass awareness program on water resource conservation and water borne disease as well as eco-friendly agricultural practices should be carried out in the Barak Valley region. Also, initiative should be taken to facilitate potable drinking water to the people residing in river catchments.

Keywords: North-east India, Barak River, fresh water habitats, microbial diversity, coliforms.

INTRODUCTION

Microorganisms consist of approximately 60% of the Earth's biomass and are highly diverse in their morphology, biochemistry as well as functional aspects (Singh et al., 2009). Despite the acknowledged value of microorganisms, our understanding of their diversity and many of their key roles in sustaining life supporting systems is still very less (Sood et al., 2010). Most of the freshwater systems in India comprise of rich biodiversity spots which needs to be explored, exploited and conserved (Johri et al., 2005).

The north-eastern region of India (22°00'N and 29°05'N; 88°00'E and 97°30'E) is considered as one of the biodiversity hotspots in the world (WCMC, 1998). River Barak is the second largest river of north-east India flowing through the Barak valley region of Assam and has a total length of 900 kilometers. Barak originates from Japvo peak in Nagaland (N 25°28' E 94°17') at (approx 3353.65 mm.s.l.) and flows through Karong village along the Manipur-Nagaland border, drains almost the entire Manipur valley before entering Assam. Then the river starts crawling east ward in the plains crossing Silchar city and receiving several other tributaries like Chiri, Jiri, Madhura, Sonai etc. It then flows through the western part of Silchar where it is joined by the river Jatinga and Dhaleswari along with several other tributaries; bifurcates into Surma-Kushiara, enters Bangladesh and joins with Ganol to form Meghna. The river Meghna then combines with Padma and falls in the Bay of Bengal (Hussain, 2012). River Barak influences the lives of all the people residing in the Barak

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Valley area but its microbial diversity is largely unknown till date due to lack of study.

In the recent past, expanding human population, industrialization, intensive agricultural practices and discharges of massive amount of wastewater into the river have resulted in deterioration of water quality. The impact of these anthropogenic activities has been so extensive that the water bodies have lost their self-purification capacity to a large extent (Sood et al., 2008). The examination of microbiological quality of river water according to technical standards is obligatory for aspects such drinking water production and irrigation or recreation since water is an important source for the transmission as well as spread of various water-borne diseases (WHO, 2007). It have been reported that in developing countries about 2.5 million deaths occurs annually due to water borne dirrhoeal diseases (Kosek et al., 2003; Obi et al., 2003; Lin et al., 2004; Obi et al., 2004). Occurrence of coliform in water samples are an indication of the presence of opportunistic bacterial pathogens like *Klebsiella, Enterobacter, Salmonella* sp., *Shigella* sp., *Vibrio cholera, Campylobacter jejuni*, *E. coli* etc. (DWAF, 1996; Grabow, 1996). Total coliform bacteria serves as system indicator in water quality testing as they directly correlate with fecal contamination (Standard Methods, 1995).

The bacteriological and physicochemical parameters of different river systems have been studied by various groups (Fokmare et al., 2001; Kavka et al., 2006; Sood et al., 2010) as these parameters largely influence the microflora of the river systems and also impose severe health hazards.

This work was undertaken with the objective to isolate bacteria from aquatic fresh water ecosystem of Barak River and its tributaries so as to assess the bacteriological contamination of the river water due to human activity.

MATERIALS AND METHODS

The water samples were collected in triplicates from various parts of River Barak and its tributaries, Assam, India (Figure 1) covering the districts of Karimganj, Cachar and Hailakandi, a total stretch of 500 km during the month of February to May, 2012. Human and animal activities are high in some of collection sites while moderate in others. The samples were collected in sterile containers and transported to laboratory for further analysis.

The physicochemical parameters such as pH, temperature and electrical conductivity (EC) of water samples were measured on the site itself using water analysis kit (Thermo-Orion water analysis kit).

For bacteriological studies, a serial dilution of each sample was made and 0.1ml of each diluted samples was plated onto nutrient agar (Hi Media) plates to determine Total Viable Count (TVC). The pH of the medium and the incubation temperature for each sample was adjusted according to the respective pH and temperature of its collection site. The results were expressed as Colony Forming Unit (CFU) per unit volume (mL), enumerated after 48 h of incubation. Depending on the variations in colony morphology or colony characteristics, the isolates were separated and stored on nutrient agar slants at 4°C for further analysis. The CFUs were calculated at 28°C and 37°C to study both the normal microflora of the rivers as well as those added from external sources, mainly due to human activity.

Qualitative analysis of the water samples was done using coliform test for which the samples were inoculated in lactose broth (Hi Media) and incubated at 37°C. From the tubes that were lactose positive, a loop full of culture was streaked in Eosin Methylene Blue (EMB, Hi Media) agar and incubated at 37°C for 24 h. Lactose fermenting and lactose non fermenting colonies were isolated separately and further characterized for identification of the isolates.

All the studies were carried out in triplicates and average was calculated for final results. The isolates were identified using standard morphological, cultural, biochemical and physiological characteristics as per the *Bergey's Manual of Determinative Bacteriology.* The data were also analyzed statistically by using t-test to compare the relative extent of pollution in River Barak and its tributaries at a confidence interval of 5%.

RESULTS

The pH of the water samples ranged from 6-7.5. The pH of the water samples collected from River Barak at Berrengaghat and River Barak at Sadarghat showed an acidic pH while the samples from River Dhaleswari at Panchgram showed an alkaline pH. The average temperature of the river water varied between 12°C to 26°C and the conductivity ranged from 60-150 µS/cm (Table 1).

At 28°C, the sample collected from Sadarghat showed highest Total Viable Counts (TVC) whereas at 37°C, TVC was maximum for the sample from Badarpurghat. At 37°C, all the samples, except the one from Katakhal, were found to have a higher TVC than those prescribed in Bureau of Indian Standards (BIS, 1991). Of the total 14 samples, 71.42% showed the presence of coliform while only 28.58% were coliform negative (Table 1).

The isolates were identified based on the morphological and biochemical characterization as per *Bergey's Manual of Determinative Bacteriology*. A total of 23 bacterial isolates were identified which comprised of the following 8 genera *Pseudomonas*, *Staphylococcus*, *E.coli*, *Enterobacter*, Sarcina, *Serretia*, *Klebsiella* and *Shigella* (Figure 2). The most abundant bacterial isolate was *Pseudomonas*, followed by *Staphylococcus* and *E.coli* while the least abundant bacteria were *Klebsiella* and *Serretia*. The t-test showed a p-value of t stat (0.9918) at

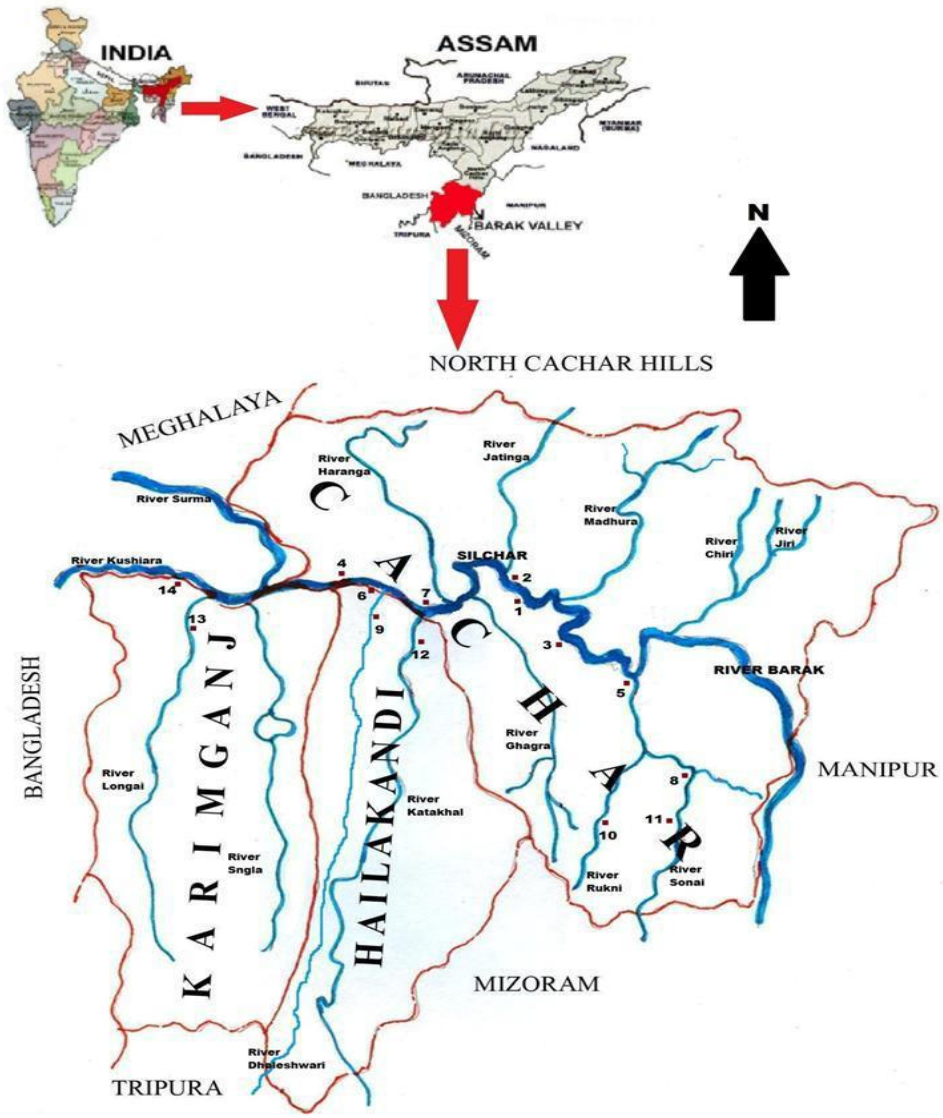

Figure 1. Map of the study area showing sample collection sites: 1. R. Barak at Sadarghat, 2. R. Barak at Annapurnaghat, 3. R. Barak at Berrengaghat, 4. R. Barak at Badarpurghat, 5. R. Barak at Sonabarighat, 6. R. Barak at Panchgram, 7. R. Barak at Katakhal, 8. R. Sonai at Sonai Bazar, 9. R. Dhaleswari, 10. R. Rukni, 11. R. Sonai at Palonghat, 12. R. Katakhal, 13. R. Longai, 14. R. Kushiara. ("R" implies "River")

Table 1 Physicochemical and bacteriological profile of the water samples

sample Collection sites[a]	Physicochemical studies			Bacteriological studies		
	pH	Temperature (°C)	Conductivity (µS/cm)	TVC (CFU/ml)		Coliform test
				At 28 °C [b]	At 37 °C [c]	
1	6.0	28.5	223	$2.6X10^7$	$8X10^5$	positive
2	7.0	25.0	254	$2.3X10^5$	$1.8X10^6$	positive
3	6.0	27.3	290	$1X10^5$	$1X10^6$	positive
4	6.5	19.0	265	$3.4X10^6$	$5.8X10^9$	positive
5	7.0	12.1	310	$1.4X10^5$	$2.8X10^7$	positive
6	6.7	14.5	366	$2.4X10^6$	$5.2X10^7$	negative

Table 1 Continue

7	7.0	23.9	345	Nil	Nil	negative
8	6.4	21.0	286	$5X10^5$	$4.2X10^7$	positive
9	7.4	16.9	324	$1.05X10^6$	$1X10^7$	positive
10	7.0	23.8	362	$1.1X10^5$	$1.1X10^6$	negative
11	7.0	15.6	301	$2X10^6$	$3.05X10^7$	positive
12	7.0	17.8	359	$1.9X10^5$	$1.6X10^6$	negative
13	7.5	18.0	289	$2.1X10^5$	$1.09X10^7$	positive
14	7.0	29.0	274	$1.01X10^6$	$8X10^6$	positive

[a] sample collection sites are numbered as per figure 1
[b] normal freshwater microflora
[c] transient microflora from external sources

Table 2. Data of t-Test (Two-Sample Assuming Equal Variances) calculated for CFUs at 37°C

	Main Barak River	*Tributaries of Barak*
Mean	840514285.7	20600000
Variance	4.78304E+18	8.3112E+14
Observations	7	7
Pooled Variance	2.39194E+18	
Hypothesized Mean Difference	0	
df	12	
t Stat	0.991808247	
P(T<=t) one-tail	0.170434698	
t Critical one-tail	1.782287556	
P(T<=t) two-tail	**0.340869396**	
t Critical two-tail	2.17881283	

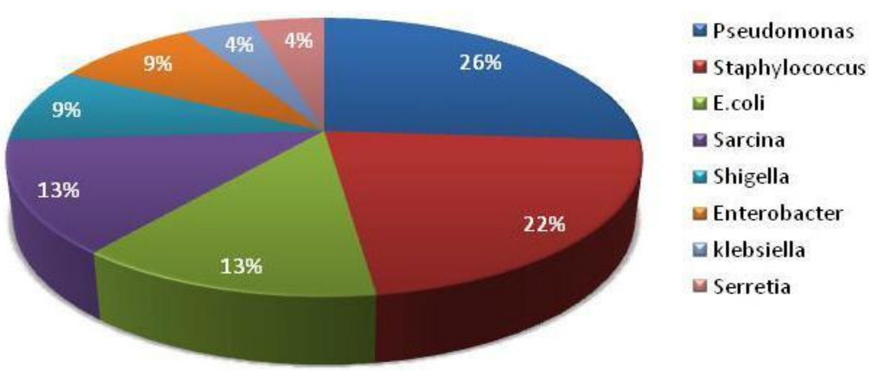

Figure 2: Rate of prevelence of various isolates in the water samples.

12 df is 0.3408, which is greater than 0.05. Therefore, it is concluded that there is no significant difference in the CFUs between main Barak River and its tributaries (Table 2).

DISCUSSION

The pH range stipulated by World Health Organization (WHO) for drinking and domestic purposes is 6.5–8.5 (WHO, 1993). The pH of a few of the studied water samples was slightly acidic otherwise water from most of the collection sites showed well buffered nature of the river water. The change in pH of the river water may be attributed to eroded soil, disposed idols as well as municipal and agricultural wastes including fertilizers, pesticides etc. Water temperatures ranged from 12°C to 29°C which are well within the temperature ranges experienced in the Barak Valley region during the period of the year (February-May) when sample collection was done. A typical river free of pollution has an average conductivity value of about 350 µS/cm (Koning & Roos, 1999) and the conductivity of majority of the studied water samples was below this value thereby indicating pollution.

The present study revealed a high TVC at 37°C in most of the water samples except that from Katakhal. The samples from River Barak at Badarpurghat showed maximum TVC and this can be attributed to the location of the site (lower-stretch) as well as a number of human activities such as bathing, washing, sewage disposal, agricultural activities etc. River Barak at Sadarghat is a waste disposal site where municipal wastes along with idols are disposed off regularly, so it also showed a high bacterial load, mainly at 28°C. Absence of bacteria in water of Katakhal can be due to lack of human activities nearby the collection site or it might even be due to the presence of some inhibitory compounds such as fungal antibiotics. Microbiological studies on the river Ganga and Gomti (India) and Densu river (Ghana) also showed high microbial loads (Sood *et.al.,* 2008; Srivastava et.al,2010; Karikari et.al 2006). Untreated water from River Barak and its tributaries are used by rural population everyday although the high TVC suggests that this should not be practised.

WHO considers water as no risk to human health if the faecal coliforms counts/100 ml is zero (WHO, 1987) but maximum of the water samples indicated presence of coliform including some pathogenic genera such as *E. coli*, *Enterobacter, Staphylococcus* etc. Isolation of such potential pathogens from river water shows that the situation with respect to water quality is alarming in River Barak and its tributaries and use of this water may transmit infectious water borne diseases. Normal freshwater microflora such as *Serratia* and *Sarcina* were present in moderate numbers. Statistical analysis using t-Test indicated that the main Barak River as well as its tributaries is polluted to the same extent thus indicating an alarming situation that needs to be addressed.

CONCLUSION

Pollution in River Barak and its tributaries is mainly due to human activities such as disposal of municipal and agricultural wastes, discharge of sewage and industrial effluents, disposal of idols and crematory activities, bathing and washing etc. Other sources of pollution include defecations and urination by domestic and wild animals, recreational activities like swimming and fishing, recurring floods etc. River Barak and its tributaries serve as a water source for a bulk of Barak Valley population and even though the urban population receives treated water for domestic use, several villagers residing along the river catchment uses river water directly for their day-to-day activities and hence more prone to water borne diseases. Also, this river and its tributaries serve as sources of fresh water fishes and polluted water hampers the aquatic ecosystem to a large extent thereby hampering the fish productivity. Release of chemical fertilizers and organic wastes is leading to problems of eutrophication and biomagnification which needs to be addressed. For sustainable management of the water resource, sanitation programmes and mass education on water conservation as well as water borne diseases should be implemented in the Barak Valley region. Also, farmers should be trained on using eco-friendly agricultural practices so as to avoid the incidence of high nutrient loads in surface waters and to enhance better management of the agrochemicals. Furthermore, water treatment plants should be managed and monitored properly so that clean and safe potable water can be provided to the both the rural and urban population of Barak valley region.

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