

## Assessment of Bacterial Pollution in Sediment of Padma River, Rajshahi, Bangladesh

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### Abstract

Padma is one of the largest rivers of Bangladesh, which has huge potential for fishery resources. Microbial pollution status of this riverine ecosystem has not been evaluated yet. Therefore, the present study was conducted to find out the bacterial pollution in sediment of this river. Four distinctive study locations were evaluated for three respective seasons in 2016. Enumeration of total heterotrophic bacteria (THB), total coliform (TC), fecal coliform (FC) and *Vibrio cholerae* were done by standard plate count technique from surface layer of sediment samples. Results revealed that all the samples were contaminated with THB, TC, FC and *Vibrio cholerae* with a range of  $20.60 \times 10^6 \pm 1.41$  cfu/g (Site-2 Padma Garden) to  $3.46 \times 10^6 \pm 0.47$  cfu/g (Site-4 Talaimari),  $140.00 \times 10^4 \pm 22.63$  cfu/g (Site-2 Padma Garden) to  $2.88 \times 10^4 \pm 0.97$  cfu/g (Site-1 T-Dam),  $5.98 \times 10^3 \pm 0.36$  cfu/g (Site-2 Padma Garden) to  $1.04 \times 10^3 \pm 0.08$  (Site-4 Talaimari) and  $3.85 \times 10^4 \pm 0.26$  cfu/g (Site-3 I-Dam) to  $0.91 \times 10^4 \pm 0.07$  cfu/g (Site-4 Talaimari), respectively. The bacterial density data had seasonality showing very high density during summer and winter seasons and the lowest in monsoon season. During the study period, it was observed that Site-2 (Padma Garden) and Site-3 (I-Dam) was comparatively more polluted with bacteria than other two sites. As per the criteria of sediment quality evaluation, the sediment of Padma River can be categorized as moderate to moderately high polluted with total heterotrophic bacteria, while very little polluted with fecal coliform bacteria. Therefore, the present study suggests a regular monitoring and assessment for reducing the bacterial load into the Padma River ecosystem through appropriate measures.



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### Introduction

Microbiological contamination of aquatic ecosystem is a major issue worldwide. Human and animal fecal material is generally considered as greater risk to human health, as it is more likely to contain human enteric pathogens. These pathogens may sometimes pose serious health-hazards to the environment and human population through water and sediment<sup>1,2</sup>.

Padma is one of the largest and important rivers in Bangladesh, which flows adjacent to Rajshah city. Therefore, a huge amount of domestic and municipal sewage is regularly mixed with its water, which ultimately pollutes it. The domestic sewage contains fecal materials originated from human and other animals. These pollutants reduce the amount of oxygen in the water and adversely affect its biodiversity<sup>3</sup>. The increased agricultural and industrial activities are now a threat to the biodiversity of Padma River due to the explosion of population growth. In such situation, evaluation of microbial pollution of this river is an urgent need of the hour. The large diversity of microorganisms in aquatic ecosystem sometimes makes it difficult to identify due to the absence of consistent and low-cost detection technique. For the convenience of identification of pathogenic bacteria, total coliforms and fecal coliforms bacterial indicators were used worldwide<sup>4</sup>. Sometimes, survivability of Coliform

bacteria in clean water for a limited time makes them a reliable indicator of organic pollution<sup>5</sup>. Thus, for routine monitoring, Fecal Indicator Bacteria (FIB) such as total coliform and fecal coliform are usually used to evaluate the contamination level of water. Sediments of aquatic environments may sometimes constitute a reservoir for FIB. It is also known that FIB survives better in sediment than the surface water<sup>6,7</sup>. Together with FIB, assessments of *Vibrio cholerae* also bears immense potential, as it is pathogenic in nature and are frequently present in aquatic ecosystem. Therefore, the aim of the present study was to evaluate the variation of these indicators (total heterotrophic, total coliform and fecal coliform) and pathogenic bacteria (*V. cholerae*) in the surface sediment of Padma River to investigate the bacterial pollution of this river.

### Materials and Methods

#### Selection of Study Area

The present study was conducted at four different study sites along the Padma River at T-Dam (Site-1), Padma Garden (Site-2), I-Dam (Site-3) and Talaimari (Site-4) covering most of the parts of Rajshahi City Corporation along the bank of the Padma River (Fig. 1). Geographical and topological characteristic of the study sites is shown in Table 1. Sampling was done in three consecutive seasons *viz.* summer, monsoon and winter in the year 2016.

**Table 1: Sampling sites, sampling code and topological characters of study sites**

Sampling station	Sampling code	Coordinates	Topological characters
T-Dam	Site-1	Latitude: N-24°21'42.41'' Longitude: E-88°34'31.18''	Discharge of effluent from some household garbage, no human activities except recreational activities.
Padma Garden	Site-2	Latitude: N-24°21'42.30'' Longitude: E-88°35'52.44''	Direct discharges of effluent from vegetable markets and slaughter discharges; discharge from household septic tanks, more human activities as recreational site.
I-Dam	Site-3	Latitude: N-24°21'34.95'' Longitude: E-88°36'39.92''	Direct discharges of effluent from household septic tanks, more human activities as recreational site.
Talaimari	Site-4	Latitude: N-24°21'29.30'' Longitude: E-88°37'30.55''	No human activities and no source of discharge into the river

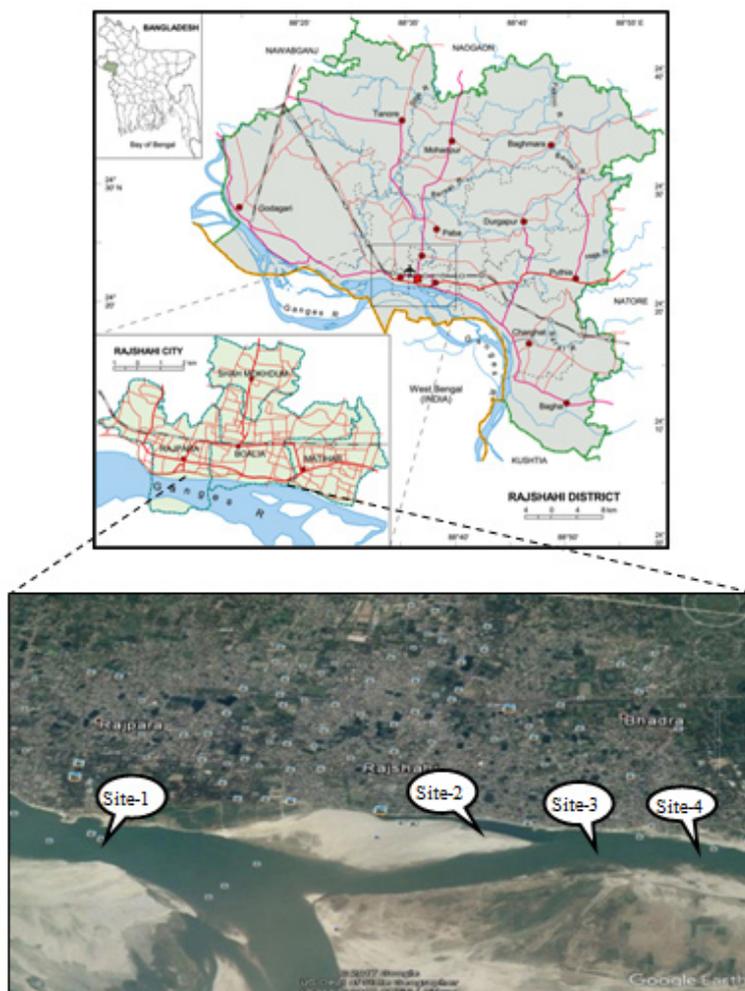


Fig. 1: Map showing locations of study sites along Padma River, Rajshahi City, Bangladesh

### Bacteriological Analysis

For the bacteriological analysis, surface layer of sediment (upto a depth of 10 cm) was sampled from the respective sampling points. The samples were delivered to laboratory in an ice box containing melting ice as quick as possible i.e. the time gap between sampling and analyses was below 3 hrs. During each sampling period, a triplicate samples were collected and bacterial analysis was done from this triplicated samples and average value for each triplicates were recorded. A total of 12 samples were collected in each time and in all, a total of 36 samples were taken for the analysis in this study. Total variation for field sampling and laboratory analysis of bacteria samples were estimated by collecting replicates for approximately 20% of samples. Precision up to 25%

relative standard deviation (RSD) log transformed was considered acceptable for all the four types of bacteria. Enumeration of Total Heterotrophic Bacteria (THB), Total Coliform (TC), Fecal Coliform (FC) and *V. cholerae* of sediment were done by Standard Plate Count (SPC) technique<sup>8</sup>. For serial dilution of samples, 1 g sediment was weighed and transferred in a sterile test tube containing 9 ml of phosphate buffer solution. A vortex (Model iSwix VT) was used to shake the tube properly for homogenization of samples. The sample was diluted to give 1:10 or 10<sup>-1</sup> dilution. Each dilution (an amount of 0.1 ml) was then transferred into the petri dish containing the respective bacterial growth media. To avoid too high load of debris and sand particles on the plate, samples were centrifuged and let to settle for

sometimes and then the aliquots of the supernatant were used for inoculation in media plates. Bubbles arising during plating of culture media were removed by lightly flaming the plate with bunsen burner. Incubation of the culture plates was done at 37 °C for 24-48 hrs and final counts of colonies were done

by a digital colony counter (Model SC5). Results were recorded as colony forming unit (cfu/g). The specific culture media and their resulted colony colours used for quantitative count of bacteria are shown in Table 2.

**Table 2: Culture media and colony coloration for quantitative bacterial analysis**

Bacterial Type	Media	Incubation Period	Colony Coloration
Total heterotrophic bacteria	Nutrient agar		Not specified
Total coliform	MacConkey agar		Pink
Fecal coliform	Eosine Methylene Blue (EMB) agar	37 °C for 24-48 h	Green metallic sheen
<i>V. cholerae</i>	Thiosulphate Citrate Bile Salts (TCBS) agar		Yellow

### Statistical Analysis

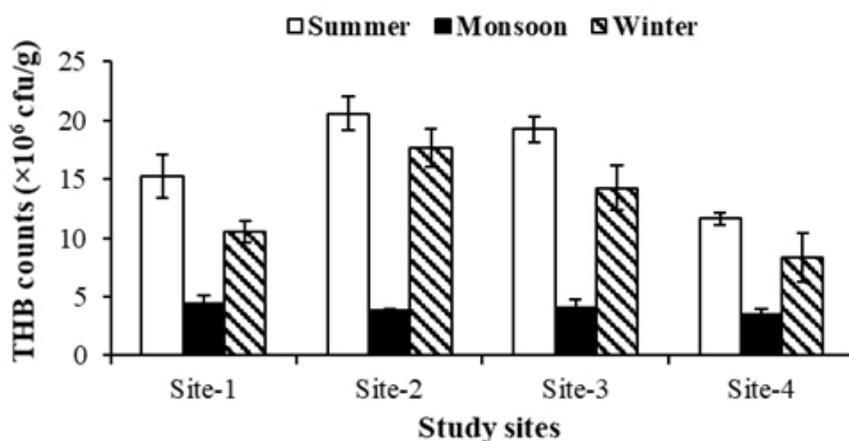
The data was analyzed using SPSS software (Version 20.0) and Microsoft Excel (Version 10). Correlation coefficient test (*r*) was used to determine the relationship between bacterial parameters separately. Correlation was considered significant at 0.01 levels (2-tailed).

### Results

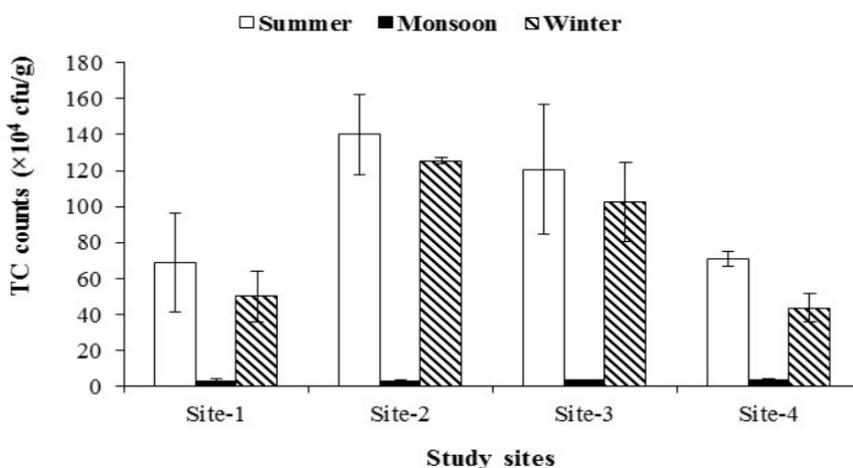
The population of Total Heterotrophic Bacterial (THB) in sediment of the Padma River over the three seasons is presented in Fig. 2. THB count was highest at all the sampling sites during summer, followed by winter and monsoon seasons. THB

count was also more uniform at all the sampling sites during monsoon season, where fluctuation was observed during other seasons (summer and winter). However, the maximum count of THB was noted at Site-2 ( $20.60 \times 10^6 \pm 1.41$  cfu/g) during summer and minimum count at Site-4 ( $3.46 \times 10^6 \pm 0.47$  cfu/g) during monsoon (Fig. 2).

Total coliform (TC) count also showed peak population during summer season at all the study sites, followed by winter season. The highest TC count was observed during summer at Site-2 ( $140.00 \times 10^4 \pm 22.63$  cfu/g) and lowest at Site-1 ( $2.88 \times 10^4 \pm 0.97$  cfu/g) during monsoon season (Fig. 3).



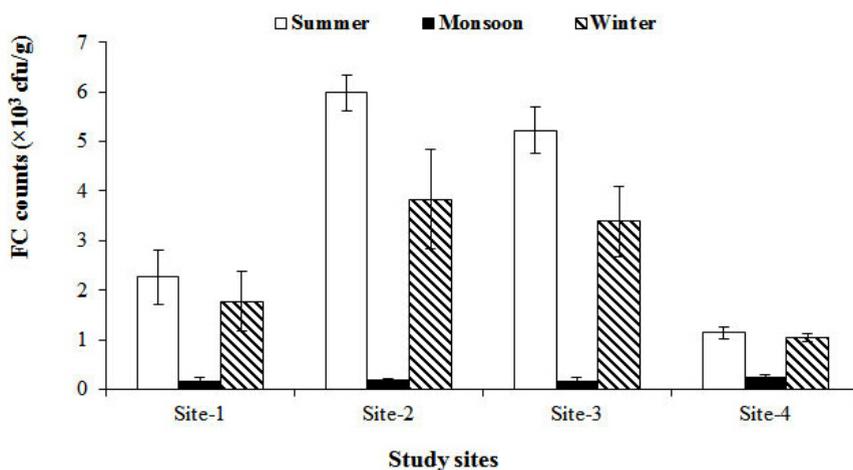
**Fig. 2: Variation of THB counts at sites during different seasons**



**Fig. 3: Variation of TC counts at sites during different seasons**

The Fecal coliform (FC) count was also fluctuated during summer and winter seasons, it was more uniform during monsoon season at all the study sites. During summer, the highest count of FC was documented at Site-2 ( $5.98 \times 10^3 \pm 0.03$  cfu/g)

and lowest at Site-4 ( $1.14 \times 10^3 \pm 0.11$  cfu/g). During winter season, the FC count ranged between  $1.04 \times 10^3 \pm 0.08$  to  $3.84 \times 10^3 \pm 1.00$  cfu/g at Site-4 and Site-2, respectively (Fig. 4).



**Fig. 4: Variation of FC counts at sites during different seasons**

The uniform distribution of *Vibrio cholerae* (VC) count was observed during monsoon season at all the sampling sites. During summer, its highest count was noted at Site-3 ( $3.85 \times 10^4 \pm 0.26$  cfu/g), followed by Site-2 ( $3.45 \times 10^4 \pm 0.05$  cfu/g). During winter season, the highest count was also found at Site-3 ( $2.48 \times 10^4 \pm 0.54$  cfu/g) and the lowest count at Site-4 ( $0.91 \times 10^4 \pm 0.07$  cfu/g) (Fig. 5).

Correlation analysis among bacterial parameters of sediment is shown in Table 3. Significant positive correlation was observed between THB and TC ( $r = 0.978$ ), THB and FC ( $r = 0.951$ ), THB and VC ( $r = 0.959$ ), TC and FC ( $r = 0.951$ ), TC and VC ( $r = 0.939$ ) and between FC and VC ( $r = 0.974$ ) at  $p < 0.01$  level respectively.

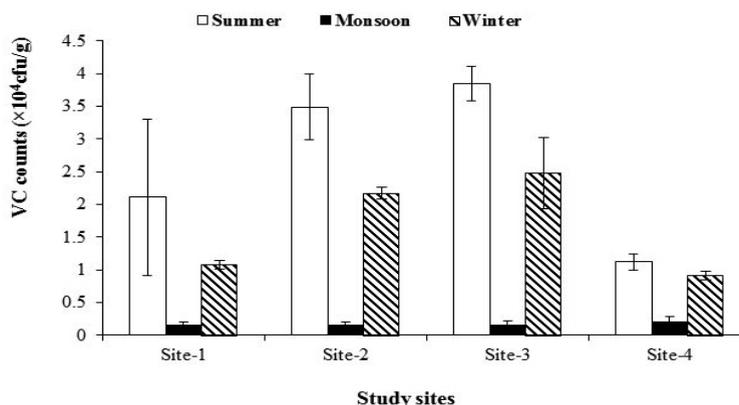


Fig. 5: Variation of FC counts at sites during different seasons

Table 3: Correlation (r) between bacterial parameters of sediment of Padma River (N = 12)

	THB	TC	FC	VC
THB	1			
TC	0.978**	1		
FC	0.951**	0.951**	1	
VC	0.959**	0.939**	0.974**	1

Key: THB = Total heterotrophic bacteria; TC = Total coliform; FC = Fecal coliform and VC = *V. cholerae*; N = No. of samples; \*\*. Correlation is significant at 0.01 level (2-tailed)

Evaluation of microbial quality of sediment of Padma River is shown in Table 4. According to the sediment quality guidelines<sup>9,10,11</sup>, the sediment of Padma River was moderately polluted with THB during monsoon at Sites-1, 2 and 3, while its moderate pollution was noted during monsoon as well as winter season at Site-4. Moderately high population of THB was found at all the sites during summer and winter seasons except winter season at Site-4. In case of FC, very little contamination was observed during monsoon season at all the study sites, while little contamination of sediment of Padma River with FC was observed at all the sites during summer and winter seasons, respectively.

**Discussion**

Municipal, industrial wastewater and agricultural runoff are main sources of pollution of river ecosystem. These polluting sources may vary from different locations and different seasons. With the

prompt development in agriculture, urbanization, and industrialization, contamination of river water is becoming a common phenomenon in Padma River. In such a grave situation, this present study is an important finding to know the present status of microbial pollution level in this river.

THB count was highest at Site-2 ( $20.60 \times 10^6 \pm 1.41$  cfu/g) during summer and lowest at Site-4 ( $3.46 \times 10^6 \pm 0.47$  cfu/g) during monsoon season. It was found that seasonal variation is one of the major factors in determining bacterial abundance in Padma River, which agrees with findings of<sup>12</sup>, who found that in tropical countries the seasonal variation is a driving force in distribution and abundance of microorganisms. Sediment quality a guideline was also showed similar findings and it indicated that during dry months the sediment of Padma River was moderately polluted with THB. Followed by THB, total coliform (TC) count was highest during summer at Site-2 ( $140.00 \times 10^4 \pm 22.63$  cfu/g) and lowest at Site-1 ( $2.88 \times 10^4 \pm 0.97$  cfu/g) during monsoon. In general, the higher counts of coliform bacteria are attributed to rapid growth of population in the open defecation areas along the river banks. Along with open defecation, the release of municipal and industrial effluents and the drainage of agricultural land fertilization contribute to the increase in concentrations. In a study conducted by<sup>13</sup> in well water to compare the survival of organisms indicated that coliform bacteria have a half-life of 17 hrs where they confirm the effectiveness of coliforms as indicators for the suitability of water. Therefore, maximum counts of bacterial population during summer season indicated worsen condition

of river water, and it may be related to the discharge of household wastes and fecal matters that directly enters into the river. However, sometimes open defecation along the river bank contributes to the above process. The disposal of city waste are mostly taken place through the drains located at Site-2, which might be a possible reason for higher total coliform at this location. On the other hand, as the growth of bacteria in an aquatic environment is

influenced by seasonal variation of temperature<sup>14</sup>, lowest count of total coliform during winter period might be due to low temperature, which is not favourable for duplication of bacterial population. Fecal pollution was initiated by the presence of raw and partially decomposed sewage materials; therefore it is a best indicator for the assessment of fecal pollution in an aquatic environment<sup>15</sup>.

**Table 4: Evaluation of sediment quality of Padma River at study sites during different seasons<sup>9, 10, 11</sup>**

Bacteria	Number of bacteria	*Sediment quality		Site-1			Site-2			Site-3			Site-4			
		S	M	S	M	W	S	M	W	S	M	W	S	M	W	
THB (x 10 <sup>5</sup> cfu/g)	0.50															
	> 0.50-1.00															
	> 1.00-10.00		√			√									√	
	> 10.00-50.00			√			√									√
	> 50.00-100.00															
	> 100.00-750.00															
	> 750.00															
FC (x 10 <sup>3</sup> cfu/g)	0.10-1.00	√														
	> 1.00-10.00	√														
	> 10.00-100.00															
	> 100.00-500.00															
	> 500.00-1000.00															
	> 1000.00-10000.00															
	> 10000.00															

Key: THB = Total heterotrophic bacteria; FC = Fecal coliform; No. of bacterial range for \*According to sediment quality guidelines: THB, 1 = 0.50, 2 = > 0.50-1.00, 3 = > 1.00-10.00, 4 = > 10.00-50.00, 5 = > 50.00-100.00, 6 = > 100.00-750.00 and 7 = > 750.00. No. of bacterial range for FC, 1 = 0.10-1.00, 2 = > 1.00-10.00, 3 = > 10.00-100.00, 4 = > 100.00-500.00, 5 = > 500.00-1000.00 and 6 = > 1000.00-10000.00 and 7 = > 10000.00; degree of loading with easily decomposable organic substance and feces: 1- very little, 2 - little, 3 - moderate, 4 - moderately high, 5 - high, 6 - very high, 7 - extremely high. S = Summer, M = Monsoon, W = Winter.

The highest value of FC was also recorded at Site-2 ( $5.98 \times 10^3 \pm 0.36$  cfu/g) in summer and the lowest at Site-3 ( $0.16 \times 10^3 \pm 0.08$  cfu/g) in monsoon during the study period. Site-2 was characterized as a high sewage and municipal disposal location which contributed to the higher fecal coliform level as reported by<sup>16</sup>, high bacterial counts are attributed to contamination by domestic sewage in the Densu River of Ghana. However, sometimes direct defecation and urination by wild and domestic animals also contaminate the surface water of river<sup>17</sup>. Higher concentrations of total and fecal coliforms were also strongly associated with rainfall and pollutant sources<sup>18</sup>. However, the concentration of fecal coliform was higher during summer season might be the reason of low water level, high organic matter and optimum growth supporting nutrient. This finding was also supported the result of sediment quality evaluation. According to sediment quality guidelines, sediment of Padma River was very little polluted with FC during wet months, while it was little polluted with the same bacterial profile during dry months. In the present study, the value of bacterial population drops down during the rainy season, which was due to the dilution caused by the rain water through flood water. Similarly, *V. cholerae* was highest at Site-3 ( $3.85 \times 10^4 \pm 0.26$  cfu/g) in summer, and lowest at Site-1 and Site-3 ( $0.15 \times 10^4 \pm 0.06$  and  $0.15 \times 10^4 \pm 0.07$  cfu/g) during monsoon season. The possible reason for higher *V. cholerae* during summer days might be due to low water level, and mounting sewage contamination, which is in agreement with an earlier findings of 19 who conducted a study on seasonal variations of *Vibrio cholerae* (non-O1) from California coastal waters. Therefore, people living along the Site-2 and Site-3 were exposed to high risk of THB, TC, FC and *V. cholerae* by their use of the surface water of Padma River especially in dry season. However, the present bacterial pollution can causes ecosystem degradation that might be devastating for ecological life of Padma River.

Correlation analysis among bacterial parameter of sediment indicates significantly ( $p < 0.01$ ) positive correlation, which means all the bacterial populations were related with each other and might possibly being originated from same sources. Therefore, addressing issues concerning major point source pollution by identifying and implementing ecologically-sound treatment processes can ensure the better management of the surface water of Padma River. In such a situation, the government can implement Ecological Sanitation (EcoSan) as a sustainable approach which can also promote proper use of waste materials as fertilizer to agriculture<sup>20</sup>.

### Conclusion

In this study, sediment of Padma River was assessed to evaluate the level of bacterial pollution. The result of the present study indicates that the pollution of sediment was categorized as moderate to moderately high with THB and very little to little polluted with FC bacteria according to sediment quality guidelines. This indicates that the river sediment is obviously polluted by indicator and pathogenic bacteria especially at Site-2 and Site-3 during the dry months. Therefore, the present study recommends to tighten the control on the discharged wastes into the river from local households and other water polluting industries and to take proper pretreatment measures of these pollutants before discharging these into the river system.

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