

BACTERIAL AND CHEMICAL POLLUTION LEVEL OF THE RIVER TURAG, GAZIPUR, BANGLADESH

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Abstract

The river Turag is the upper tributary of the river Buriganga in Bangladesh. Aerobic heterotrophic bacterial count of the river in nutrient agar (NA) and peptone yeast extract glucose (PYG) agar media ranged from 2.91×10^5 to 27.5×10^5 cfu/ml and from 1.09×10^5 to 19.3×10^5 cfu/ml, respectively. Enteric and related bacterial count on MacConkey agar was between 0.88×10^3 and 20.8×10^3 cfu/ml. Out of 91 isolated bacterial isolates, 30 were selected based on their distinctive colony morphology for detailed study. Among these, 16 were Gram-positive (53%) and 14 were Gram-negative (47%). *Bacillus* was dominant among the Gram-positive isolates (69%). The remaining were *Brochothrix thermosphacta* (6%), *Caryophanon* sp. (19%) and *Renibacterium salmoninarum* (6%). Fourteen Gram-negative, enteric and related isolates were identified as *Plesiomonas shigelloides* (43%), *Alcaligenes denitrificans* (22%), *E. coli* (14%), *Proteus* (14%) and *Citrobacter freundii* (7%). Biological oxygen demand (BOD) and chemical oxygen demand (COD) values were about 9 and 5-fold higher than standard values whereas dissolved oxygen (DO) of the river was found to be six times less than the standard value. The maximum average TDS, conductivity and turbidity was recorded during the summer season as 441.75 ± 15.52 mg/l, 858.25 ± 19.57 μ S/cm and 79.53 ± 6.49 NTU, respectively. Chemical contents *viz.* sulphate, chloride and fluoride were found to be satisfactory from pollution point of view. Both BOD and COD values along with the abundance of different heterotrophic and enteric bacteria clearly indicated that the river Turag was polluted with organic, chemical and bacterial pollutants.

Introduction

Dhaka, the capital and most populated city in Bangladesh is now a member of mega city family in the world. Rapid and unplanned urbanization, commercial development along with population pressure have made it an environmentally polluted city in the world⁽¹⁻²⁾. In Bangladesh, about 80% of all diseases are associated with waterborne

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pathogen. About 300,000 children under five die of diarrhoeal diseases every year, of which one third of the deaths occurs in the city slums and squatter settlements⁽³⁾. Pathogenic bacteria that have been transmitted by water or waste water include *Salmonella*, *Campylobacter*, *Vibrio cholerae*, *Leptospira* and *Yersinia*.

Dhaka metropolis is bounded from northwest side by the river Turag, northeast side by Baloo and Sitalakkhya and extreme south side by Buriganga. The catchment of the river Turag is occupied by nearly one third of the total population of Dhaka. There are many industries dotting the banks of this river which dispose their industrial waste into the river that pollute the water of the river to a dangerous level. Concentrations of Cd, Pb and Cr were found higher in different stations in the course of Buriganga, Sitalakhya and Turag rivers⁽⁴⁾. In the recent past, sporadic information on some physicochemical variables including the concentration of some heavy metals on the river Turag has been published⁽⁵⁻⁶⁾. Studies on the pollution aspects of this kind of water are very significant because these settlements use this water for various domestic purposes⁽⁷⁾.

Therefore, the present study was undertaken to determine the aerobic heterotrophic and enteric bacteria in the context of biological pollution level along with the physicochemical properties of the river Turag to reveal an overall status of pollution of the river Turag.

Materials and Methods

Sampling sites and collection of water samples: The water samples were collected from four selected sites (T-1 to T-4) of the river Turag, Dhaka, Bangladesh (Fig. 1) in three different seasons considering possible sources of pollution. Water samples were collected in plastic bottles sterilized with alcohol.

Bacteriological analysis: Nutrient agar (NA) and peptone yeast extract glucose (PYG) medium were used for the enumeration and isolation of aerobic heterotrophic bacteria present in samples while MacConkey agar (Oxoid) medium was used enteric bacteria. The pH of the media was adjusted to 7.2 before sterilization. Two different techniques *viz.* serial dilution plate⁽⁸⁾ and membrane filtration were used for the enumeration and isolation of bacteria. The inoculated plates were inverted and incubated at 37°C for 24 hrs in an incubator (Mettler GmbH + Co Kg 8540 Sehwabach). After 24 hrs of incubation the plates with well discrete colonies were selected for counting. Using colony counter (Digital colony counter, DC-8 OSK 100086, Kayagaki, Japan) the developed colonies were counted.

Discrete bacterial colonies were isolated immediately after counting. Further selection of the isolates was made on the basis of their different colony morphology. For provisional identification of bacteria, important biochemical tests were carried out *viz.* carbohydrate fermentation, catalase test, deep glucose agar test, tyrosine degradation, egg-yolk lecithinase test, casein hydrolysis, gelatin hydrolysis, starch hydrolysis, Kligler's

iron agar (KIA) test, levan test, methyl red test, nitrate reduction test, oxidase test, indole production test, utilization of citrate and propionate, urease production test, Voges-Proskauer (VP) test etc.⁽⁹⁻¹²⁾. Bergey's Manual of Systematic Bacteriology (Vol. 2)⁽¹²⁾ was followed for the identification of Gram-positive aerobic heterotrophic bacterial isolates. On the other hand, enteric bacteria were identified by using standard method following Manual for Laboratory Investigations of Acute Enteric Infections⁽¹³⁾ and Bergey's Manual of Systematic Bacteriology (Vol. 1)⁽¹⁴⁾.

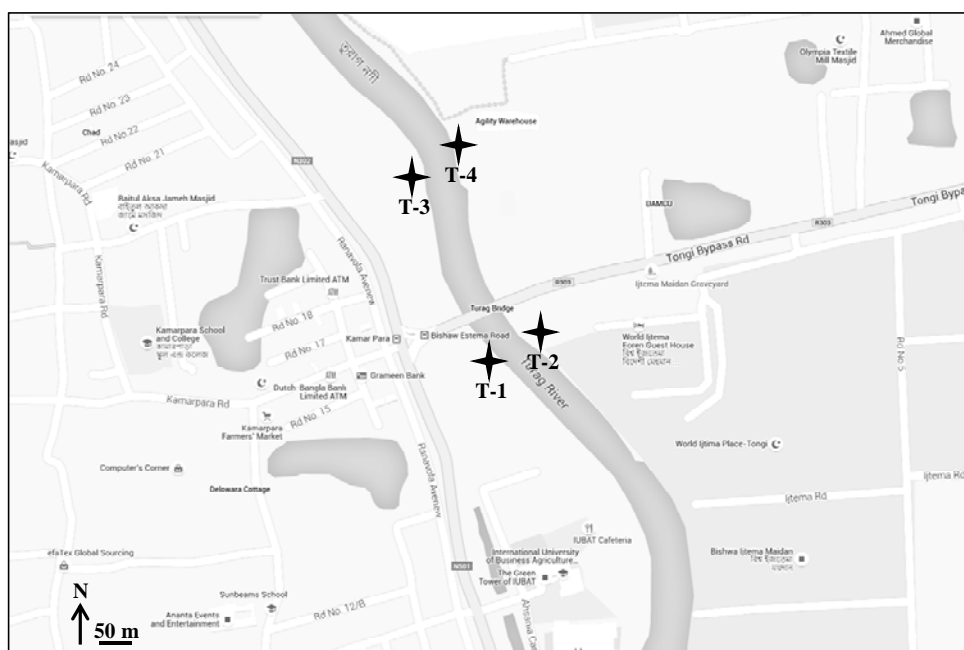


Fig. 1. Map showing different sampling sites from the river Turag. T-1: Kamarpara bridge, T-2: Ijtema field, T-3: Rusadia Gudar quay and T-4: Mill gate.

Determination of physicochemical properties: The pH of collected water samples was measured in the laboratory by glass electrode using pH meter (HM-31P, DKK-TOA Corp., Japan)⁽¹⁵⁾. Temperature of the sample water was measured at the time of water sampling with the help of a thermometer. For chemical analysis, samples were filtered (Whatman No. 42, England) to eliminate suspended solid particles. Dissolved oxygen of water was determined by DO meter (HACH HQ 30d). Biochemical Oxygen Demand (BOD₅) of water samples was determined by Respirometric Method (APHA 5210D) using BOD Sensor Set (HACH BOD TRACK II). Chemical Oxygen Demand (COD) of water samples was determined by closed reflux, titrimetric method (APHA 5220C) using certified HACH COD vials and COD Reactor (ECO 8, VELP, Italy). Total Dissolved Solids (TDS) and conductivity of the collected samples were determined using multi

range conductivity meter (HACH Sension 156 Conductivity meter). Turbidity of the water samples was determined using Turbidity meter (MODEL- 2100 Q, HACH, USA) followed by APHA 2130B⁽⁸⁾. Sulphate content of water samples was determined by turbidimetric method⁽¹⁶⁾. Chloride and fluoride contents of the water samples were determined by Ion Chromatography method (Dionex ICS-1600 Thermo Scientific, USA).

Statistical analyses: The data were analyzed to determine the descriptive statistics *viz.* statistical mean and standard deviation (SD) with SPSS v.16.0 for windows (SPSS, SAS Institute Inc. Cary, USA).

Results and Discussion

Bacterial count of the water samples of the river Turag revealed substantial number of aerobic heterotrophic bacteria (Table 1). The highest count (27.5×10^5 cfu/ml) was found in the site T-4 (Mill gate) station in the late autumn season on NA medium while the lowest count (1.09×10^5 cfu/ml) was found in the site T-1 (Kamarpara bridge) in the rainy season on PYG medium. The bacterial counts among the replicates were found to be varied and the maximum mean aerobic heterotrophic bacterial count ($13.63 \pm 9.10 \times 10^5$) was observed in the late autumn season. Enteric and related bacterial count on MacConkey agar ranged between 0.88×10^3 and 20.8×10^3 cfu/ml (Table 1) and the highest count was found in the sample of site T-4 (Mill gate) in the summer season. The maximum mean enteric and related bacterial count ($17.33 \pm 3.06 \times 10^3$) was also observed in the summer. The results demonstrated that the river Turag is considerably polluted with bacterial population. Garnier *et al.*⁽¹⁷⁻¹⁸⁾ and Hasan *et al.*⁽¹⁹⁾ reported more or less similar results showing the load of heterotrophic and nitrifying bacteria in the river and sewage lagoon. The US Department of Interior, Federal Water Pollution Control Administration in its 1968 report on water quality stated the tolerable limit for recreation purposes up to 10 cfu/ml of total and 2 cfu/ml of fecal coliforms. In a different study, Saha *et al.*⁽²⁰⁾ observed the aerobic heterotrophic bacterial count of the river Buriganga ranged from 1.0×10^5 to 42.0×10^5 cfu/ml while enteric bacterial counts MacConkey agar ranged from 0.3×10^3 to 3.5×10^4 cfu/ml.

During this investigation, of the initial 91 isolates, 30 were selected on the basis of their distinctive colony morphology and purified for detailed study. Table 2 shows important biochemical tests for identification of the bacteria isolated from the river Turag. Out of the 30 isolates, 16 were Gram-positive and remaining 14 were Gram-negative. Among the Gram-positive isolates, all were rod shaped and 11 isolates were spore former and members of the genus *Bacillus* (69%) and the remaining 5 were identified as *Brochothrix thermosphacta* (6%), *Caryophanon* sp. (19%) and *Renibacterium salmoninarum* (6%). Under *Bacillus* there were 6 distinct species *viz.* *B. acidocaldarius*, *B. coagulans*, *B. globisporus*, *B. lentus*, *B. pumilus* and *B. stearothermophilus*. The result clearly indicated that (Fig. 2) among the Gram-positive bacteria, spore former *Bacillus*

was the dominant genus (69%). All the 14 Gram-negative isolates were short rods and non-spore former. Out of them, 6 isolates (43%) were identified as *Plesiomonas shigelloides*, 3 isolates (22%) were *Alcaligenes denitrificans*, 2 isolates (14%) were *Escherichia coli*, 2 isolates (14%) belonged to the genus *Proteus* and 1 isolate (7%) was identified as *Citrobacter freundii*. *Plesiomonas* was found to be dominant genus among the Gram-negative bacteria in the river Turag. The coliform group of bacteria in general and *E. coli* in particular, was found to be universal indicator of fecal contamination⁽²¹⁾. Saha *et al.*⁽²⁰⁾ reported the presence of *Bacillus*, *Planococcus*, *Enterobacter*, *Alcaligenes*, *Salmonella*, *E. coli* and *Plesiomonas* in the river water of Buriganga, Dhaka.

Physicochemical properties of the water of the river Turag are given in Table 3. During the rainy season, river Turag merges with vast areas of floodplain on both of its banks whereas in dry season the river becomes narrow. The pH ranged from 6.90 to 7.56. The mean value in summer as recorded in the present investigation (6.99 ± 0.08) is more or less similar to that of Sikder *et al.*⁽⁶⁾. Water temperature ranged from 22.0 to 31.0°C whereas similar data (20.82 - 30.50°C) is observed by Khondker and Abed⁽²²⁾. Both pH and temperature of the river Turag remains favorable for bacterial growth all throughout the year.

Khondker and Abed⁽²²⁾ reported DO concentration of the river Turag as 0.45 - 13.3 mg/l but in the present study it ranged in between 0.21 and 0.99 mg/l. According to Bangladesh Gazette⁽²³⁾, standard values for DO should be 6 mg/l. So, the average DO value of the river Turag is approximately six times less than the standard value. Due to presence of oxygen demanding pollutants like organic wastes, there may have been a rapid depletion of dissolved oxygen from water. According to United State Public Health (USPH) standard, 5 and 4 mg/l value of BOD and COD, respectively indicate the quality for domestic and drinking water. The normal range of BOD for good water quality is 5 - 6 mg/l and COD is 6 - 10 mg/l⁽²⁴⁾. In this investigation BOD and COD values of the water samples ranged in between 29 and 65 mg/l and 31 and 79 mg/l, respectively. Maximum average BOD (49.50 ± 15.29 mg/l) was found in the summer and maximum average COD (64.03 ± 5.74 mg/l) was found in the late autumn season. In comparison with the standard values, both BOD and COD of the river Turag were found to be 9 and 5 times higher than the standard. High BOD and COD values indicated that water of the river Turag was considerably polluted with organic and chemical pollutants.

The maximum average total dissolved solids (TDS), conductivity and turbidity of the river Turag were recorded during the summer season and these were 441.75 ± 15.52 mg/l, 858.25 ± 19.57 μ S/cm and 79.53 ± 6.49 NTU, respectively. In a study, Khondker and Abed⁽²²⁾ Turag with a clear peak concentration (around 900 μ S/cm) in April, which is somewhat similar to the data acquired in this piece of work (clear peak concentration 870 μ S/cm). However, the mean conductivity of the river water is said to be increased by 7% in a recent study⁽⁶⁾. According to Bangladesh Gazette⁽²³⁾, standard values for industrial

Table 1. Bacterial count of water samples of the river Turag in different seasons and at different locations.

Seasons	Sampling site	Aerobic heterotrophic bacteria (cfu/ml) on			Enteric and related bacteria (cfu/ml) on	
		NA	PYG	Average \pm SD	MacConkey agar	Average \pm SD
Rainy season	T-1	3.30×10^5	1.09×10^5	$4.19 \pm 3.89 \times 10^5$	2.01×10^3	$2.56 \pm 1.79 \times 10^3$
	T-2	13.1×10^5	2.82×10^5		5.08×10^3	
	T-3	4.14×10^5	1.37×10^5		2.26×10^3	
	T-4	5.57×10^5	2.14×10^5		0.88×10^3	
Late autumn	T-1	4.82×10^5	3.01×10^5	$13.63 \pm 9.10 \times 10^5$	3.09×10^3	$8.07 \pm 6.61 \times 10^3$
	T-2	23.5×10^5	14.1×10^5		17.8×10^3	
	T-3	10.9×10^5	5.90×10^5		5.24×10^3	
	T-4	27.5×10^5	19.3×10^5		6.14×10^3	
Summer	T-1	2.92×10^5	2.62×10^5	$2.91 \pm 0.43 \times 10^5$	18.6×10^3	$17.33 \pm 3.06 \times 10^3$
	T-2	3.81×10^5	2.73×10^5		13.7×10^3	
	T-3	3.14×10^5	2.38×10^5		16.2×10^3	
	T-4	2.91×10^5	2.79×10^5		20.8×10^3	

Table 2. Biochemical tests for identification of the bacteria isolated from the river Turag.

Isolate No.	Biochemical profile										Identified bacteria
	V.P.	Methyl red	Motility	Citrate	Propionate	Starch	Tyrosine	Lecithinase			
T1N1	+	-	-	+	-	+	-	+	+		<i>Bacillus acidocaldarius</i>
T3N1	+	+	+	+	-	-	-	-	+		
T4P4	-	+	+	+	+	-	+	-	-		
T1N2	+	-	-	+	-	+	-	+	+		<i>B. coagulans</i>
T1N4	-	-	+	+	-	+	-	+	+		<i>B. globisporus</i>
T1P1	-	+	+	+	-	+	-	-	-		<i>B. lentus</i>
T4P3	-	+	-	+	-	+	-	+	+		
T1P2	+	+	+	+	-	+	+	-	-		
T3P1	-	-	+	+	-	+	-	+	+		
T3P2	+	+	+	+	-	-	-	-	+		<i>B. pumilus</i>
T2N1	+	-	+	+	-	-	-	-	+		<i>B. stearothermophilus</i>
T1N3	+	-	-	+	-	+	-	-	-		<i>Brochothrix thermosphacta</i>
T2N2	+	-	+	+	-	+	-	+	+		<i>Caryophanon</i> sp.
T4N2	+	-	+	+	-	+	+	+	+		
T4N3	+	-	+	+	-	+	-	-	+		
T4N4	+	+	-	+	-	-	-	-	+		<i>Renibacterium salmoninarum</i>
T3N2	+	+	+	-	-	+	+	+	+		<i>Alcaligenes denitrificans</i>
T4N1	+	+	+	-	-	+	+	+	+		
T3P3	-	-	+	-	-	+	+	+	+		
T4M1	+	+	+	+	+	+	+	+	+		<i>Citrobacter freundii</i>
T1M3	-	+	+	-	+	+	-	+	+		<i>Escherichia coli</i>
T4M2	-	+	-	+	+	-	+	-	-		
T2P1	+	-	+	-	-	+	-	+	+		<i>Plesiomonas shigelloides</i>
T4P1	+	-	+	-	-	+	-	-	+		
T4P2	+	-	+	+	+	+	-	-	+		
T1M2	+	+	+	-	-	+	+	+	+		
T2M2	-	-	+	-	-	-	-	-	+		
T3M1	-	+	+	+	+	+	-	-	+		<i>Proteus myxofaciens</i>
T2M1	+	+	+	-	-	+	-	-	+		<i>Proteus rettgeri</i>
T1M1	-	-	+	+	+	-	-	-	+		

+ = Positive, - = Negative result.

Table 3. Physicochemical properties of the water samples of the river Turag in different seasons.

Sampling site	pH	Temp. (°C)	DO (mg/l)	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	Conductivity (µs/cm)	Turbidity (NTU)	Sulfate (mg/l)	Chloride (mg/l)	Fluoride (mg/l)
T-1	7.10	27.0	0.63	54	62	75	150	22.70	51.20	33.10	0.75
T-2	7.10	27.5	0.53	29	66	80	160	30.30	57.40	38.00	1.46
T-3	7.03	27.5	0.72	37	51	74	149	17.20	50.00	34.50	0.62
T-4	7.08	28.0	0.69	31	57	82	164	17.90	48.20	34.70	1.03
Average ± SD	7.08 ± 0.03	27.5 ± 0.41	0.64 ± 0.08	37.75 ± 11.35	59.15 ± 5.20	77.75 ± 3.86	155.75 ± 7.41	22.03 ± 6.03	51.70 ± 4.00	35.08 ± 2.08	0.97 ± 0.37
Late autumn											
T-1	7.56	22.0	0.43	63	73	212	424	39.90	54.10	36.90	0.20
T-2	7.53	23.0	0.69	32	55	211	423	41.90	55.00	36.50	0.26
T-3	7.53	23.5	0.99	53	64	227	454	38.60	58.60	42.10	0.28
T-4	7.52	24.0	0.97	46	65	245	489	35.20	61.90	46.00	0.26
Average ± SD	7.54 ± 0.02	23.13 ± 0.85	0.77 ± 0.26	48.50 ± 13.03	64.03 ± 5.74	223.75 ± 15.95	447.5 ± 31.18	38.90 ± 2.82	57.40 ± 3.57	40.38 ± 4.54	0.25 ± 0.03
Summer											
T-1	6.90	30.0	0.21	65	79	433	866	83.90	98.80	90.50	0.31
T-2	6.98	29.5	0.31	60	69	465	829	74.90	100.70	95.20	1.01
T-3	6.99	29.5	0.38	34	40	434	868	86.20	102.40	93.50	0.51
T-4	7.09	31.0	0.62	39	65	435	870	73.10	101.50	94.40	0.36
Average ± SD	6.99 ± 0.08	30.00 ± 0.71	0.38 ± 0.17	49.50 ± 15.29	63.38 ± 12.71	441.75 ± 15.52	858.25 ± 19.57	79.53 ± 6.49	100.85 ± 1.53	93.4 ± 2.05	0.55 ± 0.32

effluents particularly sulphate, chloride and fluoride were 400, 150 -600 and 1 mg/l, respectively. The result of the present study revealed that the contents of thus chemical were satisfactory from the pollution point of view as they were within the standard range (maximum level of sulphate 100.85 ± 1.53 mg/l, chloride 93.4 ± 2.05 mg/l and fluoride 0.97 ± 0.37 mg/l).

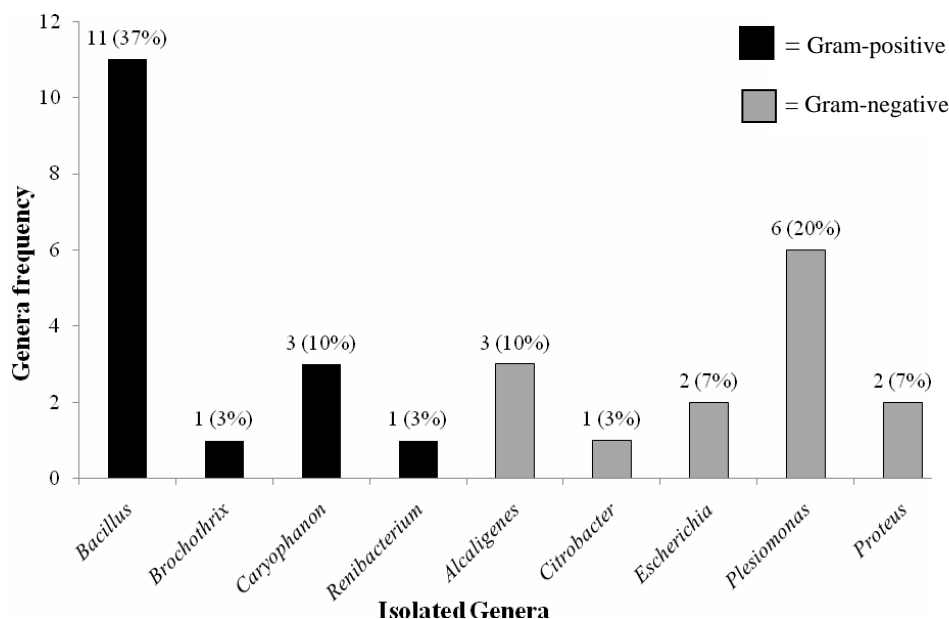


Fig. 2. Genera frequency of the identified bacterial isolates.

The load of aerobic heterotrophic bacteria and the presence and abundance of enteric and related bacteria such as *Plesiomonas*, *Alcaligenes*, *Escherichia*, *Proteus* etc. in the water clearly showed significant level of microbial pollution of the river. On the basis of DO, BOD and COD values, the river Turag water was found to be polluted. Fecal contamination via increasing anthropogenic activities in the catchment could be the main reason for it. Well managed waste disposal system should be practiced to save the river Turag from the pollution. Whereas more detailed study would be needed especially on the multi-drug resistance as well as on the hemolytic activities of the isolated Gram-negative bacteria. Research should be carried out in future which will have significant role towards the gastrointestinal disease manifestation for the people who are directly associated with this river.

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