MICROBIAL RISK ASSESSMENT OF SURFACE WATER IN DHAKA CITY

This thesis is submitted to BRAC University in partial fulfilment of the requirements for the degree of Bachelor of Science in Biotechnology

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Abstract

The purpose of the research work is to examine the water quality of three major water sources of Dhaka city on basis of the presence of microbial contamination. This research has been carried out for a year and the presence of total coliform and the fecal coliform in the surface waters of Dhaka city have been measured. During the study, water samples were collected from three water bodies (Hatirjheel Lake, Gulshan Lake, and Buriganga River) during two seasons (winter and summer). Average coliform counts for single grab samples from Hatirjheel, Gulshan, and Buriganga were 3475 cfu/ml, 7112cfu/mL, 10610cfu/mL respectively during summer and 15860 cfu/ml, 9990 cfu/mL, 45400 cfu/mL respectively during winter. Similarly, average fecal coliform counts for single grab samples from Hatirjheel, Gulshan, and Buriganga were 390cfu/mL, 1352cfu/mL, 362cfu/mL respectively during summer and 447cfu/mL, 183cfu/mL, 923cfu/mL respectively during winter. While total coliform counts were higher during summer, fecal coliform contamination was generally higher during winter in these water bodies. Overall, all three water bodies were heavily polluted with counts of fecal indicator bacteria being two orders of magnitude higher than the maximum permissible limit for water bodies to be used for recreation purpose. Therefore, this study highlights the importance for implementing mitigation strategies to make these water bodies safe for human activities.

Keywords:

Total Coliform, Fecal Coliform, Contamination, World Health Organization.

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Author

Declaration of Authenticity

I, the undersigned, declare that the research work embodying the results reported in this thesis entitled "Microbial Risk Assessment of Surface Waters in Dhaka" is my original work, gathered and utilized for the sole purpose of fulfilling the objectives of this study. I confirm that the work has not been previously submitted to any other institution, in whole or in part, for a higher degree or diploma. I further declare that the thesis has been composed entirely by me under the supervision of Mr. Mahbubul Hasan Siddiqee, Sr. Lecturer, Microbiology Programme, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, except where stated otherwise by reference or acknowledgement.

Abrar Nadeem

Certified

Mahbubul Hasan Siddiqee,

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List of Abbreviations **Abbreviation**	**In full**
µl	Microlitres
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ETEC	Enterotoxigenic *Escherichia coli*
icddr, b	International Centre for Diarrhoeal Disease Research, Bangladesh
MAR	Multiple antibiotic resistance
MIU	Motility Indole Urease
ml	Millilitres
MNA	Modified nutrient agar
MNB	Modified nutrient broth
MRVP	Methyl red- Voges Proskauer
Nos.	Number of
PCR	Polymerase chain reaction
psi	Pounds per square inch
RNA	Ribonucleic acid

PCR	Polymerase chain reaction
Psi	Pounds per square inch
RNA	Ribonucleic acid
*rpo*A	RNA polymerase subunit Alpha
TAE	Tris base- Acetic acid- EDTA

TCBS	Thiosulfate-citrate-bile salts-sucrose
TSI	Triple sugar iron
UV	Ultraviolet

Chapter 1

Introduction

Water occupies approximately 71% of earth surface and plays a significant role for the survival of living matter. Water is polluted by human activities. As a result, the ground water and surface water become unusable and poses a potential threat to human health. A lot of factors are considered as pollutants and the factors are a wide spectrum of pathogens, chemical waste, agricultural runoff, industrial discharge.

Water has numerous uses like drinking, industrial, irrigation, livestock, aesthetics, boating, swimming, fishing and so on. However, the scope of such uses is getting narrowed due to bacteriological contaminations. (R. Abdul Vahith, 2016).

Both the pathogenic and beneficial organism is present in the aquatic system. Microbial indicators are used to determine whether or not the water is safe for use. Recently the concerns have been raised about the appropriate use of microbial indicators to regulate recreational water bodies. Microorganisms are presence in nature in a great number and high density so that it can be used as an indicator for the suitability of water. (Sana Shafi, 2013).

Bacterial contamination has become a concern of people through media due to health issues. They are responsible to cause water borne illness which has devastated civilization for centuries. The United States Environment Protection Agency established the standard level of faecal coliform and total coliform as it functions as indicator of contamination. This identification is carried out as it is less expensive.

Water containing fecal matter has the capability to spread waterborne diseases to the fish consumers and swimmers and major economic losses to for shell fish harvesting and business

(Travette et al.,2005). Recreational waters are susceptible to variety of sources of microbial pollution, which can contain pathogenic microorganisms that causes infection in gastrointestinal tract, upper respiratory tract, ears, eyes and nasal cavity and skin (WHO. 2003).

Payment, Waite, Dufour (2003) specified some characteristics those should be applicable for coliform and they are:

- It should be present whenever the pathogen is present and unpolluted should be free from them.
- It should not have the capabilities to propagate in natural water.
- It should be available in a larger quantity to facilitate testing.
- It should react to natural environment and treatment in a similar pattern to the pathogen.
- It should be readily isolated, identified and enumerated.
- It should be cost effective for frequent testing.
- It should not be pathogenic to minimize health issue.

The earliest use of the indicator organism was carried out by Escherish, a motile and rod shaped microorganism in the fecs of new born and sucking babies that causes milk to cloth. He named it Bacterium coli commune. (Medema, 2003).

Fecal coliform bacteria exist in intestine of worm blooded animals and humans and are found in bodily waste, animal droppings and naturally in soil. Most of the fecal coliforms are comprised of *E.coli* and a serotype of *E.coli* (0157:H7).

Total coliform bacteria are relatively are collection of relatively harmless residing in the gut of both cold blooded and warm blooded animals. It is used to check whether the water disinfection works properly or not. If the temperature of the water increases up to 65 degree or above, the number of total coliform goes down in naturally contamination of water. *Enterobacter* and *Citrobacter* along with *klebshiella* are examples of total coliform are not always associated with fecal matter. Fecal coliform is a sub type of total coliform that is highly related to fecal contamination. However, later it has been found that *klebshiella* grows in same temperature as fecal matter. As a result, *klebshiella* gives false positive result indicating presence of fecal matter. To avoid this problem, presence of *E.coli* is measured to measure fecal pollution.

In order to check contamination, water samples are collected and tests are performed for the identification of indicators. After that the water source can be closed or restricted. The government and environmental department would grade the water. The presence of indicator bacteria is used to measure water quality all over the world. However, there is not such standard test to indicate the presence. Basically total coliform, fecal coliform, enterococci are enlisted indicator organisms.((R.T. Noble, 2003).

The United States Environment Protection Agency provided the definition of fresh water which will free from pathogenic microorganism, radioactivity, turbidity, undesirable taste, odor and color.

Hatirjheel is a recreational water body linked with gulshan and Banani Lake inaugurated in 2013. After the establishment of this project has become a major matter of concern to the people of Dhaka city. The filthy bangunbari khal has become the most attractive place to visit after spending nineteen hundred crore Bangladeshi taka on its development process. The aim of this project is to drain storm water which covers one third of storm of Dhaka city. Apart from that this place is used for sightseeing and transportation. Open dumping is addressed as a major problem for this project as it affects the water quality.((Jalal, 2012).

Buriganga River is a significant water body due to the transportation of goods and people. As a lot of people reside near this river, the pollution free water is an obvious demand. Some of the residents of old Dhaka is directly involved in using this water. This unconsciousness can induce potential health risk to the dwellers. The river is polluted as domestic, municipal and chemical waste is discharged in this river.

The purpose of this study was to investigate the presence of total coliform and fecal coliform in those three distinctive water bodies and the level of contamination exists there in two major seasons.

Chapter 2

Experimental Design and Methodology

2.1 Study Area:

Dhaka is the capital of Bangladesh and it is the one of the fastest growing cities Asia. It is situated in the central part of the country (UN 1999; Alam and Rabbani 2007). The surface of the city has been changed due to heavy urbanization (UN 1999). The watershed of Dhaka covers an area of approximately 1,500 km2 (IWM, 2007) including the Dhaka Metropolitan Area (260 km2) .

There are many water bodies in dhaka cities. However, this work was on three water sources and they are gulshan lake, Hatirjheel and Buriganga river).

The Buriganga River which flows near Hazaribag is one of the most polluted rivers in Bangladesh. Besides the tannery industries, many other industrial, chemical, food processing, sanitary sewage, lubricants from launch/marine vessels etc. are polluting this river. In dry season the water level goes down so it has a different number of pollution rates.

Both the gulshan and Hatirjheel are linked together. Gulshan lake is located in gulshan thana, shahajadpur and baridhara. It is a dumping zone and it is declared as ecological critical area. On the other hand hatirjheel is located in Rampura to Begunbari. It was made in order to diminish traffic congestion.

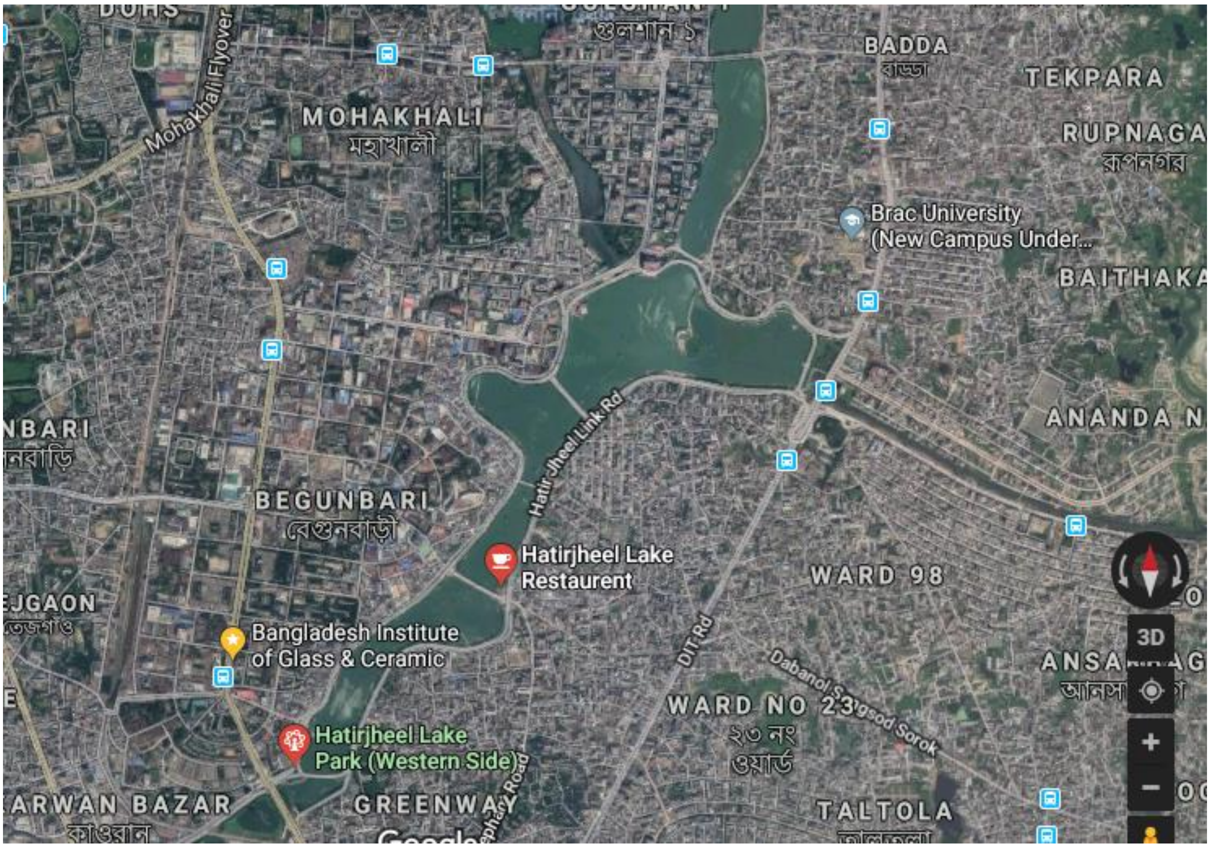


Figure 2.1 : Gulshan lake.

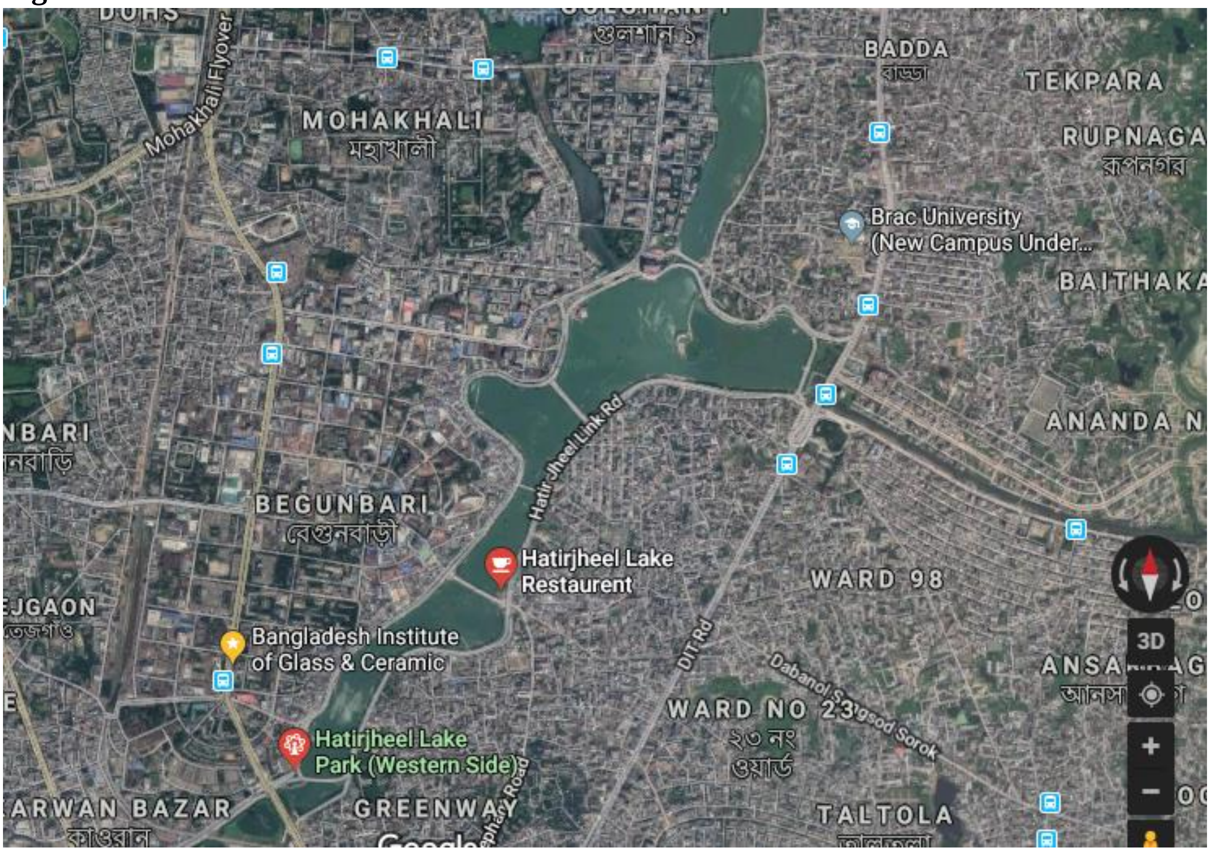


Figure 2.2 : Hatirjheel lake.

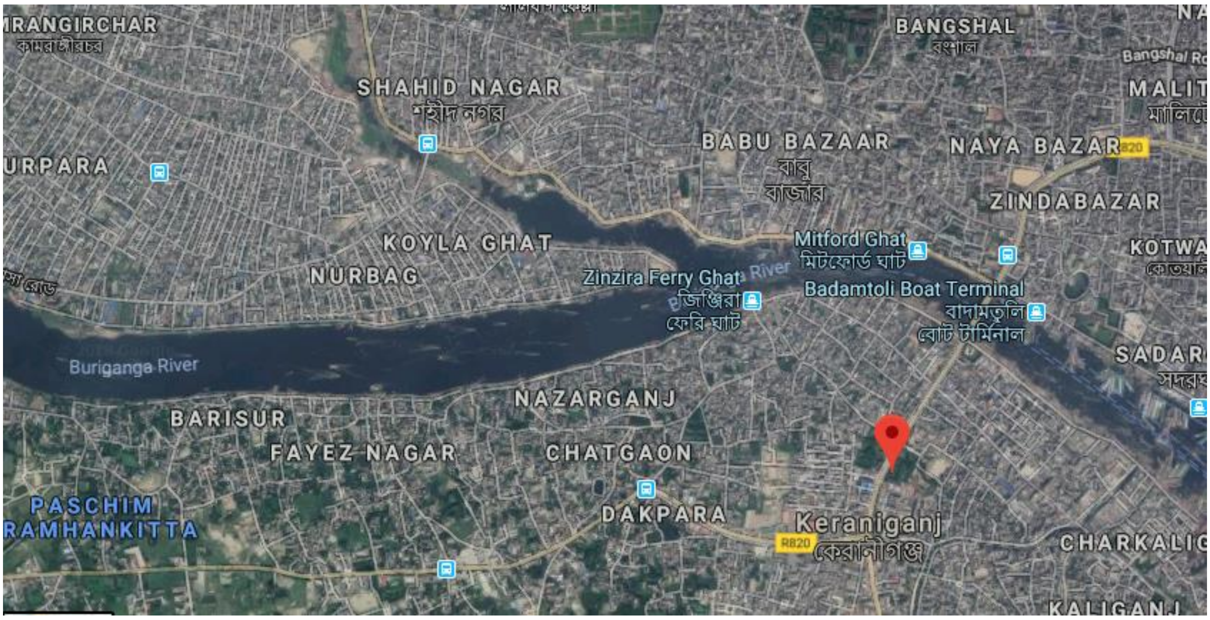


Figure 2.3: Buriganga River.

2.2 Sampling procedure:

The water was collected from Buiganga, Hatirjheel and Gulshan lake using autoclaved plastic bottles. Total 120 samples were taken from the places. Samples were collected from December 2017 to October 2018. Samples are processed within 2 hours.

2.3 Processing of samples:

Samples were used in agar plate using spread plate technique. Samples were mixed with saline water using serial dilution and 10^-1 and 10^-2 dilution factors are taken. Then they are spreaded on agar plates. There were two media; MFC for fecal coliforms and Mackonkey for total coliform. For MFC, the incubation period was 24 hours at 45 degree and for Mackonkey the incubation period was 24 hours at 37 degree celsious. After incubation, colonies are counted and cfu/ml is measured following this formula.

Colony forming unit/ml = **Number of colonies** X **Dilution Factor/ Volume Plated.**

Chapter 3

Result and Discussion

Dhaka and its municipality comprise of greater Dhaka and it has more than 18 million people and has an extremely high growth rate of population which is 4.2%. Dhaka has multiple water resources that help Dhaka to grow rapidly. However, these water bodies are becoming a burden element for the city. So, three major water bodies was taken under the microscope and to perform that total coliform and faecal coliform were determined to know the faecal contamination. In this research, total 170 samples were taken from three water bodies. All the samples were categorized into two types on the basis of the prominent seasons; winter and summer.

In order to analyze faecal contamination, total coliform and faecal coliform are identified. The presence of total coliform in gulshan, Hatirjheel and Buriganga are 7112 cfu/ml, 3475 cfu/ml, 10610 cfu/ml in winter. To contrast, 9990 cfu/ml, 15860 cfu/ml, 45400 cfu/ml are got found in those water reservoirs. In the case of faecal coliform, the colony forming units per ml are 1352, 390, 362 in gulshan lake, Hatirjheel lake and Buriganga respectively in winter. Whereas in summer the cfu's were 183, 447, 923 . From this data it is evident that Buriganga has the highest and Hatirjheel has the lowest faecal pollution rate in both instances.

Total coliform and faecal coliform comparing with United States Environment Protection Agency Standard:

Total 170 samples are analyzed and it is found that most of samples have crossed the permissible level of cfu's in water. The water of these lakes poses a potential health threat to the people. According to standard, faecal contamination occurs if the total coliform exceeds 10000 cfu/100ml and faecal coliform reaches 400 cfu/100ml. The pollution rate is 30-450 times higher than the standard level.

Table 1 Date, Average cfu/100ml for total coliform and fecal coliform

Date(G)	AVG CFU/ML(T C)	AVG CFU/ML(F C)	Date(H)	AVG CFU/ML (T C)	AVG CFU/ML(F C)	Date(B)	AVG CFU/ML(T C)	AVG CFU/ML(F C)
14.11.2017	2750	2050	14.11.2017	3250	500	06.02.2018	2250	0
14.11.2017	5400	2800	14.11.2017	5200	1000	06.02.2018	6900	0
14.11.2017	2100	50	14.11.2017	1000	0	06.02.2018	14000	1600
14.11.2017	4400	0	14.11.2017	1050	900	06.02.2018	31300	1800
21.11.2017	3800	3050	14.11.2017	2150	300	06.02.2018	1450	0
21.11.2017	5900	650	14.11.2017	2000	0	06.02.2018	16050	1000
21.11.2017	3700	0	14.11.2017	7050	200	06.02.2018	11900	950
23.01.2018	9000	1650	14.11.2017	3250	1500	06.02.2018	6350	400
23.01.2018	3250	3650	23.01.2018	4900	100	06.02.2018	4750	0
23.01.2018	5950	3500	23.01.2018	22000	0	06.02.2018	8100	0
30.01.2018	4550	700	30.01.2018	5350	1100	12.02.2018	12550	0
30.01.2018	4350	150	30.01.2018	1250	0	12.02.2018	3800	0
30.01.2018	9650	100	30.01.2018	1200	1700	12.02.2018	3950	0
30.01.2018	18350	250	30.01.2018	2100	0	12.02.2018	5850	0
30.01.2018	5400	700	30.01.2018	2050	500	12.02.2018	37900	0
05.03.2018	11500	0	05.03.2018	600	0	12.02.2018	4300	550
05.03.2018	4800	0	05.03.2018	300	0	12.02.2018	14150	0
05.03.2018	9750	1150	05.03.2018	350	0	12.02.2018	4900	100
05.03.2018	12550	6450	05.03.2018	3000	0	12.02.2018	1900	0
05.03.2018	15100	150	05.03.2018	1450	0	12.02.2018	19850	850
21.08.2018	8900	0	21.08.2018	38050	1400	17.09.2018	67750	0
21.08.2018	9700	1600	21.08.2018	14350	300	17.09.2018	74000	0
21.08.2018	7800	0	21.08.2018	8050	0	17.09.2018	27200	0
21.08.2018	17900	0	21.08.2018	33650	0	17.09.2018	35650	0
21.08.2018	27450	0	21.08.2018	42250	0	17.09.2018	104250	0
21.08.2018	19250	0	21.08.2018	10400	0	17.09.2018	40050	100
21.08.2018	10050	100	21.08.2018	19450	0	17.09.2018	17000	350
11.09.2018	5250	150	21.08.2018	8850	0	17.09.2018	53150	750
11.09.2018	8550	0	11.09.2018	12750	1500	17.09.2018	53500	4800
11.09.2018	6150	100	11.09.2018	4250	400	17.09.2018	34500	0
11.09.2018	8450	200	11.09.2018	3100	200	17.09.2018	28250	200
11.09.2018	5350	0	11.09.2018	11550	1600	17.09.2018	43700	0
11.09.2018	4950	350	11.09.2018	11350	300	17.09.2018	13600	0
11.09.2018	9650	250	11.09.2018	19850	1000	17.09.2018	51750	0
11.09.2018	9350	1500	11.09.2018	14200	300	17.09.2018	24850	900
	277050	29200		276200	9500	25.09.2018	91650	0
	7915.714286	834.2857143		5524	271.4285714	25.09.2018	40200	100

25.09.2018	95050	0
25.09.2018	19200	50
25.09.2018	23650	4400
25.09.2018	47400	200
25.09.2018	18000	550
25.09.2018	23750	0
25.09.2018	62200	0
25.09.2018	55350	100
25.09.2018	66450	0
25.09.2018	46550	750
25.09.2018	7000	0
25.09.2018	50850	100
25.09.2018	45500	14350

(T C= total coliform, F C = faecal coliform, G= gulshan, H= Hatirjheel, B= Bruriganga)

The current United States Environment protection Agency suggests that the fecal matter will be less than 200 cfu/100ml for body contact, 1000 cfu/100ml for for fishing, 2000 cfu/100 ml for domestic water supply and 1 cfu/100 ml for drinking purpose. According to this standard, water of these lakes is not usable at any state.

Even though total coliform is not the best option to detect faecal contamination, it can keep us free from skin rashes and ear infection.(Seyfried and cook,1984). *Psudomonous geruginosa* has a positive correlation with total coliform.(Guimaraes,1993).

As the samples are taken from multiple points, variability should be checked. Due to variability check, the difference of different samples in a particular day can easily be analyzed. For example, if there is a point for the exhaustion of chemical waste, this place will have more facecal pollution. Environmental factor are responsible for the fluctuation of pollution. The determination of pollution let us know about the potential health risk if i contacts with body. For instance, if high rate of total coliform is present due to abundance of *E.coli* and somehow it is linked with septic tank, then there is a possibility of spreading waterborne illness. However, if the *E.coli* is not present, then the chance is lower to occur waterborne illness.

Seasonal Variation:

Total coliform presence in Gulshan Lake:

Even though Bangladesh has six seasons, the capital Dhaka holds two prominent seasons; winter and summer. Total 170 samples were experimented divided into two seasons. There are some factors like abundance of water, temperature, concentration of chemicals etcetera causes the variation of faecal pollution in lakes.

Total coliform in Winter	Total coliform in Summer

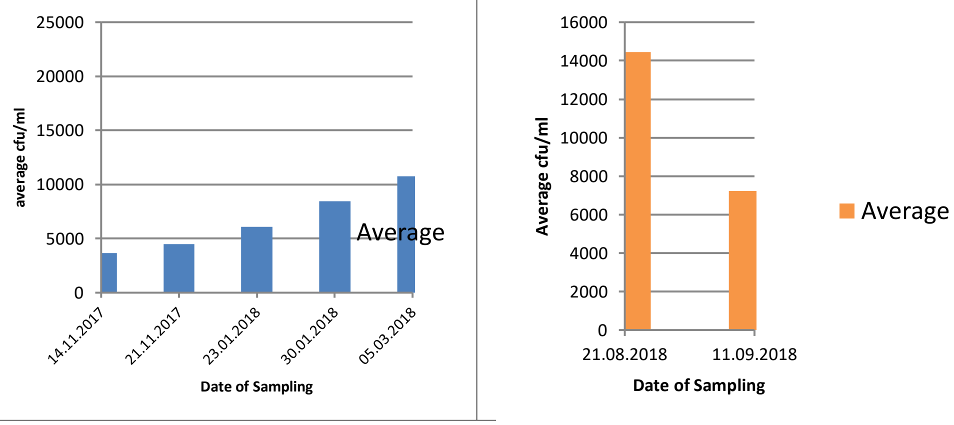


Figure 3.1: Seasonal Variation of Total coliform in Gulshan(Here X axis represents date of sampling and Y axis is for cfu/ml).

In winter, total 20 samples were analyzed from 14th November 2017 to 5th March 2018 and a symmetric trend line is visualized. The earlier samples have less variability comparing to the later ones. In summer, 15 samples were taken in 21th August 2018 and 11th August 2018. The first date of collection is more polluted with high variability comparing to last date collection. However, from graph it is resembles that summer contains more polluted water.

Total coliform in Hatirjheel:

Total coliform in Winter	Total coliform in Summer

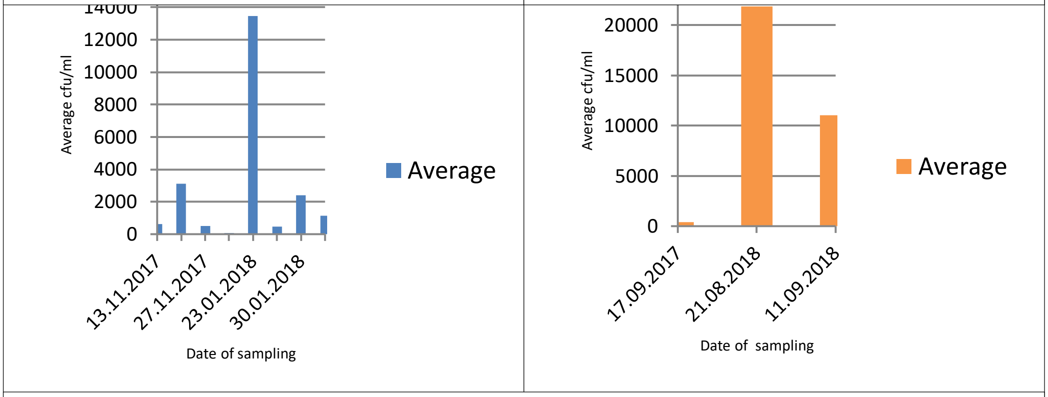


Figure 3.2: Seasonal Variation of Total coliform in Hatirjheel (Here X axis represents date of sampling and Y axis is for cfu/ml).

Total 52 samples were experimented from 13th November 2017 to 30th January 2018 in winter and an uneven growth pattern is seen in the graph and samples are variable. In summer, an unusual pattern of growth is observed with high variability and the samples are collected from 17th September 2017 to 11th September 2018.

Total Coliform in Buriganga:

Total coliform in Winter	Total coliform in Summer

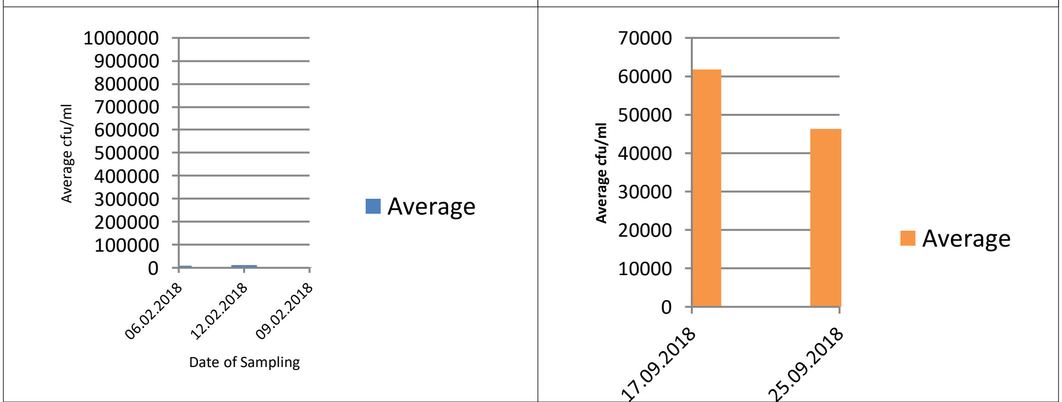


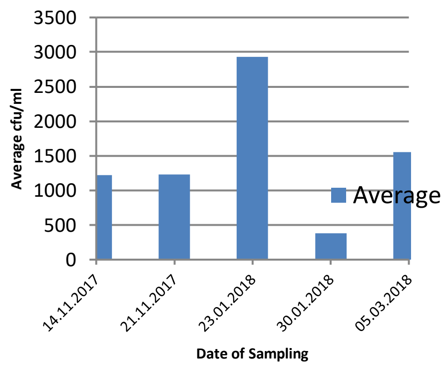
Figure 3.1: Seasonal Variation of Total coliform in Buriganga river (Here X axis represents date of sampling and Y axis is for cfu/ml).

Total 30 samples were taken from 09th February 2018 and 12th February 2018. In first two samples have similar pattern of growth and last samples have minimum growth of contamination. A high variability is observed. In summer, 40 samples are analyzed in 17th

September 2018 and 25th September 2018. Similar pattern of growth is visible. However, summer has the highest pollution rate.

Fecal coliform in Gulshan:

Faecal coliform in Winter



Faecal coliform in Summer

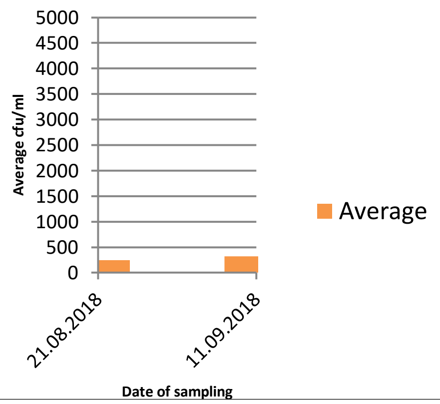
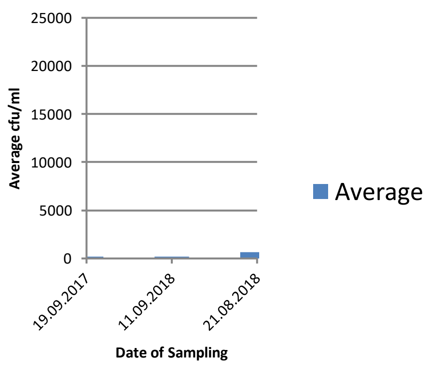


Figure 3.4: Seasonal Variation of Fecal coliform in Gulshan (Here X axis represents date of sampling and Y axis is for cfu/ml).

In winter, total 20 samples were analyzed from 14th November 2017 to 5th March 2018 and a symmetric trend line is visualized. The earlier samples have less variability comparing to the later ones. In summer, 15 samples were taken in 21th August 2018 and 11th August 2018. The first date of collection is similarly polluted with high variability comparing to last date collection. However, from graph it is resembles that winter contains more polluted water.

Fecal coliform in hatirjheel:

Faecal coliform in Winter



Faecal coliform in Summer

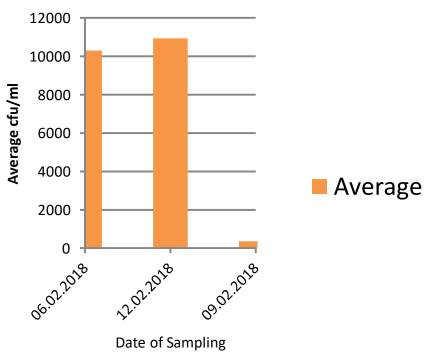
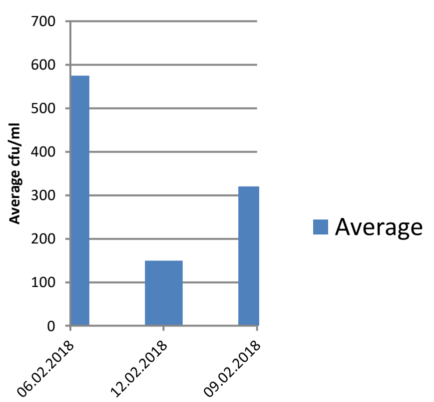


Figure 3.5: Seasonal Variation of Total coliform in Hatirjheel (Here X axis represents date of sampling and Y axis is for cfu/ml).

Total 52 samples were experimented from 13th November 2017 to 30th January 2018 in winter and a gradual growth pattern is seen in the graph and samples are variable. In summer, a similar pattern of growth is observed with high variability and the samples are collected from 17th September 2017 to 11th September 2018. In comparison summer has the highest pollution rate.

Fecal coliform in Buriganga:

Faecal coliform in Winter



Faecal coliform in Summer

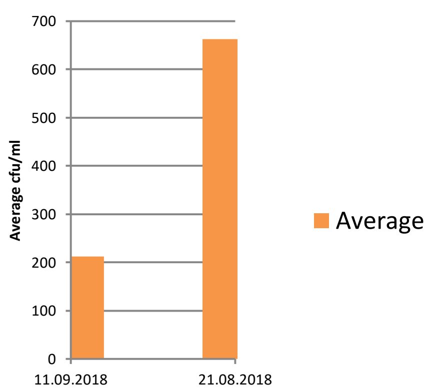


Figure 3.6: Seasonal Variation of Total coliform in Buriganga river (Here X axis represents date of sampling and Y axis is for cfu/ml).

Total 30 samples were taken from 09th February 2018 and 12th February 2018. In first two samples have similar pattern of growth and last samples have minimum growth of contamination. A high variability is observed. In summer, 40 samples are analyzed in 17th September 2018 and 25th September 2018. Samples in last day contain more fecal coliform. However, summer has the highest pollution rate comparing to winter.

Chapter 5

Conclusion

This objective of this experiment was to check the faecal pollution rate in major water contributors of Dhaka city. Therefore, they are the reservoirs of two-third storm water of Dhaka city. It is evident that the water bodies are highly affected with fecal contamination. Buriganga is the most polluted meanwhile Gulshan Lake is second and Hatirjheel holds the third position. However, all the water bodies are not in a position to use. The wastes of industries, domestic garbage and poop of hanging toilets are dumped into the river and lakes make the faecal pollution.

In order to abate or diminish the faecal pollution, the urbanization and industrialization near the water bodies must be implemented following a strict city guideline. The awareness related to this subject must be increased. The water taxi and other vehicle must be stopped without any procrastination.

The entire Hatirjheel integrated with Gulshan Lake is a multimillion dollar project. If the project becomes unproductive then it will be a significant loss for our country.

Chapter 6

Recommendations for Future Work

It is important to obtain the most accurate data and more number of sampling throughout the years will give the most credible results. This research work focused only on total coliform and faecal coliform rather identification on *E.coli* may give the prudent results. Due to faecal matter, sometimes false positive result may be observed in some organism. As a result, the severity of the water for water borne illness would not determine accurately. *E.coli* is the main reason for faecal contamination in lakes and river and identification of *E.coli* through molecular analysis will take this research into a superior level.

Chapter 7

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Appendix A: Media Composition

Mackonkey Agar:

Composition	Amount(g/L)
Peptones (meat and casein)	3
Pancreatic digest of gelatin	17
Lactose monohydrate	10
Bile salts	1.5
Sodium chloride	5
Crystal violet	0.001
Neutral red	0.03
Agar	13.5
pH after sterilization(at 25°C)	7.1±0.2

MFC Agar :

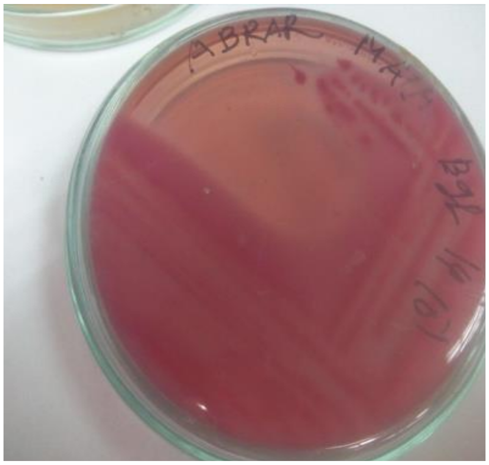
Composition	Amount(g/L)

Tryptose	10
Proteose peptone	5
Yeast extract	3
Lactose	12.5
Bile salts mixture	1.5
Sodium chloride	1.5
Aniline blue	0.1
Agar	1.5
Final pH (at 25°C)	7.4±0.2

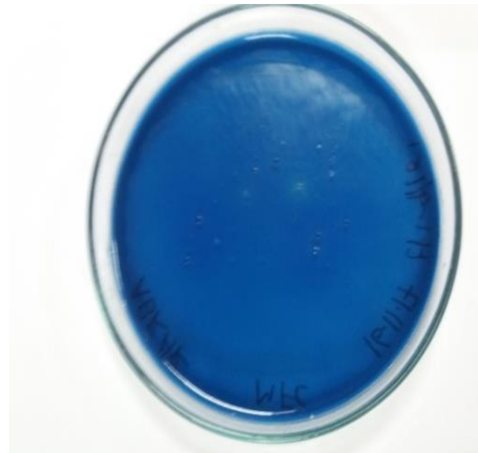
Appendix C: Instruments

Instruments	Company
Autoclave	SAARC
Cellulose filter paper(9.0 cm)	Whatman
Freeze(-20^{o}C)	Siemens
High Speed Refrigerated Micro Centrifuge, Model: MX- 307	Tomy Kogyo Co Ltd, Japan
Incubator	SAARC
Microcentrifuge tubes	Tarsons, India
Micropipette ()	Eppendorf, Germany
Micropipette (2-20 µl)	Eppendorf, Germany
Micropipette (100-1000 µl)	Eppendorf, Germany
PCR Tubes	Tarsons, India
pH meter, Model: E-201-C	ShaangaiRuosuaa Technology Company, China
Pipette (5 ml, 10ml)	Eppendorf, Germany
Power Supply, Model: ELITE 300 Plus	Wealtec Corp, USA
Refrigerator (4^{o}C), Model:0636	Samsung
Safety cabinet, Class II Microbiological	SAARC
Shaking Icubator, Model: JSSI-1000C	JS Research Inc, Rep. of Korea
Shaking Incubator, Model: WIS-20R	Daihan Scientific Co Ltd, Korea
Surgical Millipore syringe filter (0.22 µm)	Millex-GS
Thermal Cycler, 2720	Applied Biosystems, Singapore
UV Transilluminator, Model MO-20 312 nm	Wealtec Corp., USA
Vortex Mixer	VWR International
Water bath	Daihan Scientific Co Ltd, Korea
Weighing balance	ADAM Equipment TM, United Kingdom

Appendix D: Photographs



A. GROWTH OF TOTAL COLIFORM



B. GROWH OF FAECAL COLIFORM

C.AESHETICS OF GULSHAN LAKE

D.MOBILITY IN BURIGANGA

E. NIGHT LIFE IN HATIRJHEEL