Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Maghbazar, Rampura, Badda and Bashundhara

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June 2019

Declaration

I hereby declare that the thesis project titled “Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Maghbazar, Khilgaon, Badda and Rampura” has been written and submitted by me, Apsara Dey Jhilik and has been carried out under the supervision of Mahbubul Hasan Siddiqee, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution . I have acknowledged all main sources of help and appropriately cited through full and accurate referencing.

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Acknowledgement

First and foremost, I would like to express my thanks to my Parents because they have given me all the support during my research days. I am also thankful to God for His blessings to my daily life, good health and healthy mind. I acknowledge my esteem to Professor **A F M Yusuf Haider** and Chairperson of MNS Department and Professor **Mahboob Hossain** for allowing me and encouraging me to complete my undergraduate thesis.

My regards, gratitude, indebtedness and appreciation go to my respected supervisor **Mahbubul Hasan Siddiqee**, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and never-ending inspiration throughout the entire period of my research work. I would like to thank and express my deepest gratitude for guiding me in my report writing and providing time to time suggestions regarding setting of experimental designs, interpretation of results and subsequent directions for the whole work without being a bit of impatient. It would have been impossible to submit my report without his cordial help.

I am also grateful to the respective Lab officer and my mentor **Asma Binte Afzal** and **Md. Mahmudul Hasan** for their great support. I am extremely thankful and indebted to them for sharing expertise, and sincere and valuable guidance and encouragement extended to me.

I would like to extend my appreciation to the respective Lab officers Shamim Akhter Chowdhury for her suggestions and moral support during my work.

I also appreciate my thesis partners **Fariha, Nowrin, Shanto** and **Nowroze** for their kind cooperation and active support throughout my work.

Apsara Dey Jhilik
July, 2019.

Abstract

Dhaka is one of the most congested cities of the world, With a population of over 18 million . The existing water supply system is not enough to fulfill the demand of clean drinking water for people in Dhaka city.This study aims at detecting coliform(FC) and *Salmonella spp* in supplied and domestically treated (Boiled or Filtered) waters in Maghbazar ,Rampura, Badda and Bashundhara zones. A total of 157 samples were collected from above mentioned four residential areas starting from October 2018 to April 2019.The water samples were tested . The supplied water samples collected from these four zones produced variable FC counts (cfu/100mL); zero, 1-5, 6-30, 31- 100, and >100 were detected in 34%, 8.12%, 14.56%, 42%, and 4.78% samples respectively . While 67% of the boiled water samples yielded no FC, others showed variable degrees of FC counts (cfu/100mL); 1-5 in 10.37%, 6-30 in 13.79% and >100 count was detected in 8.32% samples. In case of the filtered waters 57% samples had zero FC counts. However, FC counts (cfu/100mL) of 1-5, 6- 30, 31-100, and >100 were detected in 1.80%, 8.37%, 6.50% and 2.50% samples respectively.The presence of *Salmonella spp* were detected by performing PCR of the water samples .Water samples collected from Maghbazar , Rampur and Badda (nine to ten samples respectively) were detected with presence of *Salmonella spp* which reveals the raw water contains (10-14) % *Salmonella spp* and treated water contains 1% *Salmonalla spp.*The result of this study suggests that, the water samples from these areas are possibly contaminated with pathogenic bacteria.

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Chapter: 1

Introduction

1.1 Importance of water

Water is one of the most essential elements for the human body, as it is present in every cell, tissue and organ of the body. A good hydration enables nutrients to be carried by the bloodstream to all cells, nourishing these structures so that they can be well-formed. It also regulates our body functions : chemical reactions, breathing, circulation, kidney functions, detoxification, digestion, defense systems, skin, in short, everything that is necessary to maintain life. Adequate water intake enables our body to excrete waste through perspiration, urination, and defecation. The kidneys and liver use it to help flush out waste, as do our intestines. We need to drink at least enough water daily to replace the water that the body normally loses through perspiration, waste removal, and other functions. Thus, the water that we drink must be pure.We drink water either by boiling or by filtering it. The quality of drinking water is a main environmental constraint in the field of health. Access to safe drinking water is the basic human right of every citizen and ensuring provision of safe drinking water is of paramount importance to protect health of the communities, however access to this basic need is a big challenge in the present century. Therewith the question comes,the water we drink in our home,is it safe enough or not.

1.2 Possibility of fecal contamination

Microbiological water analysis is a method of analysing water to estimate the numbers of microbes present and, if needed, to find out what sort of microbes they are. It is not feasible to test for all the possible microbial pathogens in water, so a single group of microorganisms that came from the same source as human pathogens is used to indicate the presence of pathogens. In 1914, the U.S. Public Health Service adopted the use of coliform bacteria as “indicator microorganisms” to indicate the

presence of fecal contamination in water. Ideally, if indicator microorganisms are detected in a substance, it indicates the presence of fecal contamination and therefore possible presence of pathogenic microorganisms in the water. Fecal coliform bacteria are present in large numbers in the feces and intestinal tracts of humans and other warm-blooded animals, and can enter water bodies from human and animal waste. If a large number of fecal coliform bacteria (over 200 colonies/100 millilitres (ml) of water sample) are found in water, it is possible that pathogenic (disease- or illnesscausing) organisms are also present in the water. When fecal coliform counts are high there might be possibility of occurrence of waterborne gastroenteritis because these indicators are associated with waterborne illness.

1.3 Possebilities of infection via Drinking water

Water generally gets to homes in one of two ways. Either it is delivered by a city/country water department DWASA (Dhaka Water supply & sewerage authority), or people supply their own water, normally from a well. It is essential to coordinate some basic prerequisites for drinking water which are providing to the dwellers and the authority should inquire it before supply such as water which supply to the consumers should be absolutely free from pathogenic microorganisms which can reason of disease, aesthetically winsome, categorically clear and the compounds which are harmful for health should be absence (Alom & Habib, 2016) .

Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is relatively easy and inexpensive to test for fecal coliform.The presence of fecal coliform bacteria indicates that the water has been contaminated with the fecal material of man or other animals. At the time this occurred, the source

water may have been contaminated by pathogens or disease producing bacteria or viruses which can also exist in fecal material.The presence of fecal contamination is an indicator that a potential health risk exists for individuals exposed to this water. Studies suggest that *E. coli* is a more reliable indicator of fecal pollution and the occurrence of pathogens in water than total and thermotolerant coliforms . Therefore, the use of *E. coli* as the main bacterial indicator instead of other coliform bacteria has been proposed in water quality monitoring programs which tailors the microbiological quality of water. Pathogens such as *Salmonella spp*, *E. coli* had been found in human and animal feces ultimately find their way into water supply through seepage of improperly treated sewage into ground water (DiPaola, 1998). Inadequate sanitation and unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005).

However, the presence and abundance of these coliform bacteria are not always correlated with the presence of human pathogens (Noble and Fuhrman, 2001) and their strength in risk assessments depends on cell abundance (EPA, 1986; FDA, 2013) and the types of pathogens present (Wade et al., 2003).The fecal indicator should satisfy all of the specific criteria, for example:

• Can be tested for easily

• Is of human or other animal origin

• Survives as long as, or longer than, pathogens

• Is present at densities correlated with fecal contamination

• Can be used as a surrogate for many different pathogens

• Is appropriate for fresh and saline aqueous environment

In order to check the contamination of water in the above mentioned residential areas,samples are collected and performed for identifying the presence of indicator organism.The 2011 WHO guidelines for drinking water give a tolerance range for *E.coli* in drinking water. Although it is preferable that drinking water contain no *E.coli*, samples containing fewer than 10 *E. coli* colonies per 100 mL sample are considered low risk. For the purpose of this study counts that exceeded 200 were capped at 200 as counting error increased significantly.

Table 1.3.1 WHO (2011) classification and color-code scheme for *E. coli* colonies per 100 mL water sample

Colour	Blue	Green	Yellow	Orange	Red
Remarks	In conformity	Low Risk	Intermediate Risk	High Risk	Very High Risk
Count per 100 ml	0	1-10	10-100	100-1000	>1000

The presence of *E. coli* at any point in the treated water indicates there is a potential health risk from consuming the water. Additional actions to be taken in these cases include notifying the responsible authorities, issuing a boil water advisory, investigating the cause of the contamination, and implementing corrective actions.The reason of this could be the degree of contamination which is associated with seasonal influences like downfall.The possibilities might become obvious during the sanitary inspection or these could be the result of cross-contamination or contamination that brought about by leaks in pipeline after sampling again.

1.4 Waterborne diseases in Dhaka

Diarrheal diseases are extraordinarily common with a worldwide distribution, and diarrheagenic *Escherichia coli (E. coli)* strains are important causative agents . Five types of diarrheagenic *E. coli* have been identified, namely Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), and Enteroinvasive *E. coli* (EIEC). The major virulence factor, which is also a defining characteristic of EHEC, is Shiga toxin (Stx). Shiga toxin-producing *E.coli* (STEC) strains produce one or both of the two major types of Shiga toxins, designated Stx1 and Stx2. Production of Stx2 is associated with an increased risk of developing the hemolytic-uremic syndrome . Sequence homologies for the A and B subunits are 55% and 57% for the prototypical Stx1 and Stx2 toxins, respectively . ETEC strains are identified by the ability to produce enterotoxins; the heatlabile toxin (LT) and the heat-stable toxin (ST)

Salmonella may be found in water sources that have been contaminated with the feces of infected humans or animals. Waste can enter the water through different ways, including sewage overflows, sewage systems that are not working properly, polluted storm water runoff, and agricultural runoff. Again wells may be more vulnerable to such contamination after flooding, particularly if the wells are shallow, have been dug or bored, or have been submerged by floodwater for long periods of time.*Salmonella* infections in humans vary with the serovar, the strain, the infectious dose, the nature of the water, and the host status. Certain serovars are highly pathogenic for humans; the virulence of more rare serovars is unknown. Strains of the same serovar are also known to differ in their pathogenicity. An oral dose of at least 10^5*Salmonella typhi* cells are needed to cause typhoid in 50% of human volunteers, whereas at least 10^9 *S. typhimurium* cells (oral dose) are needed to cause symptoms of a toxic infection. Typhoid is strictly a human disease.The incidence of human disease increases when the level of development of a country decreases (i.e. water sewage systems). Where

the hygienic conditions are missing, the probability of fecal contamination of water remains high and so is the incidence of typhoid.

Table 1.4.1 EPA National Primary Drinking Water Standards (EPA, 2002)

Microorganism	**MCLG[1] (mg/L)**	**MCL or TT (mg/L)**	**Potential Health Effects from Ingestion via Water**	**Sources of Contaminant in Drinking Water**
Cryptosporidium	as of 01/01/02: zero	as of 01/01/02: TT	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Giardia lamblia	zero	TT	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human and animal fecal waste
Heterotrophic plate count (HPC)	n/a	TT	HPC has no health effects, but can indicate how effective treatment is at controlling microorganisms.	HPC measures a range of bacteria that are naturally present in the environment
Legionella	zero	TT	Legionnaire's Disease, commonly known as pneumonia	Found naturally in water; multiplies in heating systems
Total Coliforms (including fecal coliform and *E. Coli*)[2]	zero	5.0%	Used as an indicator that other potentially harmful bacteria may be present[4]	Coliforms are naturally present in the environment; fecal coliforms and *E. coli* come from human and animal fecal waste.
Turbidity	n/a	TT	Turbidity is a measure of the cloudiness of water. It is used to indicate water quality and filtration effectiveness (e.g., whether disease-causing organisms are present). Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. These organisms can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.	Soil runoff
Viruses (enteric)	zero	TT	Gastrointestinal illness (e.g., diarrhea, vomiting, cramps)	Human fecal waste

Waterborne diseases are responsible for 24% of all deaths in Bangladesh and leads to the death over 100,000 children over year(icddr,b 2014).On average,over 250 children die everyday from a lack of clean water.Clean water is a huge concern in Bangladesh,where a substantial portion of the population lack access to improved water sources or education about the dangers of unfiltered or contaminated water(WHO 2018).Access to sanitation is also a major issue - 28 million people people in Bangladesh lack access to clean water,66 million lack access to sanitation(Brighter Dawns,2018).

Water is life, but it oftens turns into a curse for the city dwellers in Dhaka.The water supply in Dhaka depend on ground water extraction.87% of the city's total water demands are met through ground water extraction(DWASA,2011). Water supplied by Water and Sewerage Authority (DWASA) is not only inadequate but also filthy, stinky and toxic.Contamination of water can also be takes place in distribution system,tanks or reservoirs for lack of routinely monitoring.The most common category of water-borne disease is represented by diarrhea. Diarrhoeal and other gastro-intestinal diseases follow a transmission pattern that is called fecal-oral transmission. The pathogen is released into the environment with feces where it stays until finding re-entry through the oral route with contaminated water. The overall sanitation and personal hygiene standard of the community or the country thus in a large measure determines the extent of re-entry of the pathogen into the body.Hygiene remains the most lagging area.There is a real risk that the accomplishments of increased access to improved water sources and latrines will not necessarily lead to proportionate health and nutritional gains given the concerning state of hygiene practice. Poor people, mostly living in the slum areas, are being neglected both at demand and supply side and are more deprived of having acceess to potable water. Approximately 31.43 percent households in Dhaka city do not have access to piped connection and they have to rely on NGO or other sources (standpipe)(Azim uddin ,2011). Despite little consumption, they have to pay more than middle-income or high- income group people.

1.5 Selected zones of this study(Maghbazar,Rampura ,Badda and Bashundhara)

Dhaka is the capital and most populated city in Bangladesh with constant changes and adjustments because of spontaneous urbanization process. Among a lot more issues, one of the serious issues of this city is the water issue. Due to low water quality millions of Bangladeshis are at risk for waterborne diseases, including a wide variety of serious bacterial infections like typhoid and severe bloody diarrhea .Maghbazar, Badda, Rampura and Bashundhara are the four metropolitan and residential areas in Dhaka city. Beside these areas many markets, hospitals, schools, slums, restaurants, tea stalls are situated. So, it's a matter of concern that water supplied in these areas is suitable for daily work or not and whether this water is contaminated with microorganism like *Escherichia coli O157:H7* and *Salmonella spp* or not. Due to the influx of people into Dhaka city, many individuals inhabit these areas.

1.6 Aim of the study

The current study was designed to resolve the microbiological quality of household water and source water of DWASA collected from different location of Dhaka city which were mainly used in drinking and other household purpose. As the microbiological condition is very important for water quality, so one of the aim of this piece of research work was to find out the possible cause of fecal contamination that might cause severe waterborne fatal disease.

Chapter: 2

Materials and Method

2.1 Sample collection

Total 157 water samples were collected from different places of Dhaka city including (Maghbazar, Rampura, Badda, Bashundhara) from October 2018 to April 2019. The samples were aseptically collected mainly from houses and slums near the area. Samples were labeled in the field and transported to the laboratory and were processed in the Laboratory of Microbiology, BRAC University within 5 to 6 hours of collection.

2.2 Sampling sites:

Water samples were collected from Maghbazar, Rampura, Badda, Bashundhara and were analyzed.The urbanized areas of Bangladesh are expanding, and 34% of the total population lives in urban areas.The total population in Maghbazar area is 1,85,442. It is under Ramna thana and administered by the Dhaka South City Corporation. The reasons people prefer living in Dhaka South is the many prestigious educational institutions,centre of business and finance and transport system.The living condition in slum of Maghbazar area is abominable because of access to safe water, drainage and sanitation facilities. People from this area complained to WASA about dirty water supply.

Rampura Thana has a population of 138,923 and a population density of 49,615 per square kilometer. There is also a slum in Rampura Thana called Rampura Slum. Rampura pump station is responsible for providing water to the Thana. The pump draws water from Balu river, Debdholai river, and Narai river. Increasing water pollution have led to calls for adding a water treatment plant to the pump.The pump is managed by Dhaka WASA of Water Supply and Sewerage Authority. WASA has faced criticism for supplying polluted water to the Thana.

The Badda area is about 1978 acres .The total population of this area is 1,80,209 and population per density is 22556 sq/km. In these area markets, hospitals, schools, slums, restaurants, tea stalls are situated.So, it is a matter of great concern for the people who are living in this area,if the WASA water supplies are suitable for daily work and drinking purpose.

Bashundhara Residential Area is a private residential area of Vatara Thana in Dhaka District . It is owned and operated by Bashundhara Group.This area is considered as flood flow zones .Drainage problem is the main problem of Bashundhara residential area. During rainy season, rain water gets stuck on roads due to lack of proper drainage system or diffuses into the damaged water pipelines.It causes the contamination of water.Though the people of this area are provided with many facilities.

Figure : Satellite map of Badda zone

Figure: Satellite map of Bashundhara zone

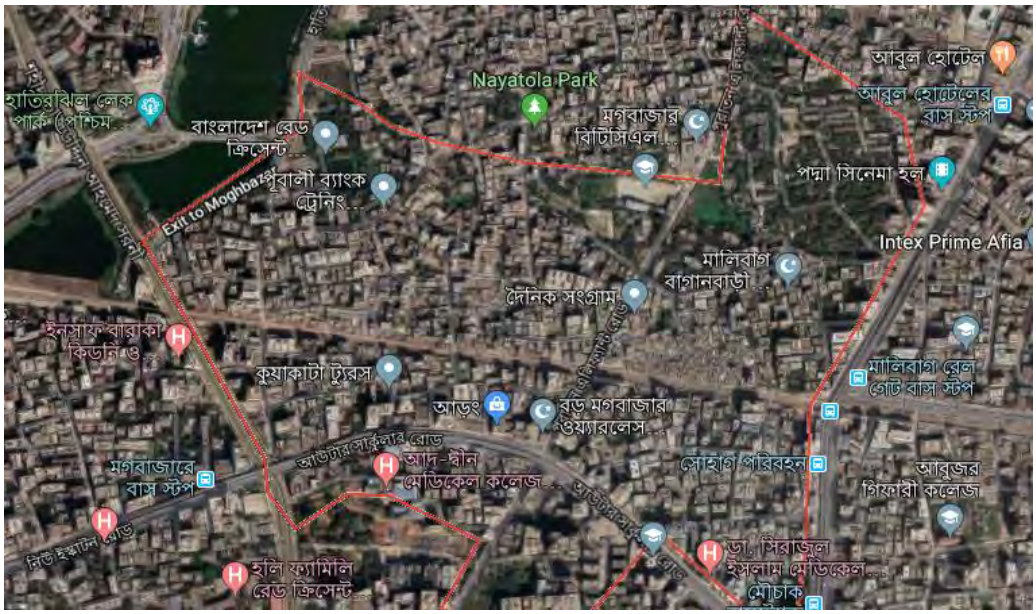

Figure : Satellite map of Maghbazar zone

Figure: Satellite map of Rampura zone

2.3 Sample processing

2.3.1 Filtration

100ml of collected sample was filtered. Filter paper was placed on m-FC plate and the plate was incubated at 44.5℃ for 24 hours. After 24 hours, the plate was observed. Only dark blue colonies were counted as they were colonies produced by *E. coli.* The colonies were identified by streaking them on EMB Agar. Only the colonies that have a green metalic sheen are *E. coli.* 100 ml of same collected sample was filtered again. Filter paper was placed in conical flask containing 50 ml of TSB supplemented with 2.5% NaCl and the flask was incubated at 37◦C for 12 hours. 300µl of autoclaved glycerol was placed in an autoclaved eppendrof. After incubation was completed, TSB was transferred to this eppendrof and vortexed. This eppendrof was stored at -20◦C. This is how the

sample was stocked. 1.5 ml of incubated TSB was transferred to an autoclaved eppendrof and centrifuged at 14,000 rpm for 10 minutes. After centrifugation the supernatant was discarded. The pellet was stored at -20℃ after the mouth of the eppendrof had been wrapped with paraflim. This is how the sample was preserved until DNA extraction was carried out.

2.3.2 DNA extraction protocol

Some microorganisms require the execution of specific steps for genomic analysis, such as in cellular DNA extraction. It is known that simply boiling a suspension of *E.coli* or *Salmonella* spp, for instance, is an effective method for inducing cellular lysis, for carrying out Polymerase Chain Reactions (PCR) (Ranjbar, Naghoni, Afshar, Nikkhahi, & Mohammadi, 2016).

After 12 hours of incubation 1.7ml of TSB were transferred to a 2ml micro-centrifuge tubes and stocked with 0.3ml of glycerol. TSB containing bacteria were transferred to micro centrifuge tubes about 1.5ml and that centrifuged for 10 minutes at 14,000 rpm (Kobayashi et al., 2009). The pellet were collected and added 400µl of distilled water that were stored at room temperature an inverted the mixture for washing. The mixtures were centrifuged for 5 minutes at 13,000 rpm and then removed supernatant. Then the pellets were re-suspended with 400µl of distilled water. The cells were lysed at 100˚C for 7 minutes. After heat shocks were performed micro-centrifuge tubes were transferred in ice for 10 minute to performed cold shock. After 10 minutes, centrifuges were performed for 5 minutes at 13,000 rpm. The supernatant contained with DNA were transferred into new micro-centrifuges tubes and wrapped with paraflim and stored at -20˚C.

2.3.2 Raw DNA gel run

The supernatant was subjected to gel run to check for the presence of DNA. Preparation of primers for PCR (stock solution and working solution)

Table 2.3.2 Sequences of primers used for amplification by PCR.

Primer name	**Sequence (5'-3')**	**Product (bp)**	**Target**	**Reference**
Salmonella-F	GTATTGTTGATTAATGACATCCG	403	*invA*	(Ranjbar, Naghoni,
Salmonella-R	ATATTACGCTACGGAAACACGTT	403	*invA*	Afshar, Nikkhahi, & Mohammadi, 2016)

bp=base pair,PCR= Polymerase chain reaction

Stock solution of *Salmonella* forward primer was prepared by adding 317µl of PCR water with 0.22mg of oligo (primer powder) for 100µM [concentration]. Stock solution of *Salmonella* reverse primer was prepared by adding 302µl of PCR water with 0.21mg of oligo (primer powder) **f**or 100µM [concentration]. Working solution of *Salmonella* forward and reverse primer was prepared by adding 900µl of PCR water with 100µl of stock solution for 100µM [concentration]

2.3.4 Preparation of control for PCR

Reference bacterial strains *Salmonella typhi* were streaked onto selective Medias XLD agar and incubated for 24 hours at 37ºC. After incubation, single colonies were picked and inoculated in LB broth. This was incubated for 24 hours at 37ºC. After incubation LB containing micro-centrifuge tubes were centrifuged for 10 minutes at 14,000 rpm. The pellets were collected. These pellets were used for DNA extraction. After DNA extraction, gel electrophoresis was carried out to observe if DNA were extracted from these samples properly. After conformation PCR was performed.

2.3.5 PCR

PCR assay were performed in tubes with a total volume of 25µl. The reaction mixtures commonly contained nuclease free water 5µl, forward primer 2.5µl, reverse primer 2.5µl, template 5µl. And master mix 10µl. Pipetting was done in careful manner so that no bubbles present and perform spinning. After the initial preparation was taken, PCR were performed under the following conditions: 35 cycles with initial denaturation at 94˚C for 5 minutes heat denaturation at 95°C for 30 seconds, primer annealing at 60°C for 30 seconds, and DNA extension at 72°C for 60 seconds and final extension at 72˚C for 8 minutes in micro-centrifuge tubes gradient master cycler. Sterile water was used instead of template DNA to provide a negative control to monitor the contamination of external DNA in the PCR reagents in PCR reaction.

2.3.6 Gel electrophoresis

Conventional agarose gel electrophoresis was performed to confirm that the PCR reaction amplified the correct target gene. The amplified DNA were separated by 1% agarose gel electrophoresis at 70 voltages, stained with ethidium bromide, and visualized by UV trans- illuminator. 1500 base-pair of DNA ladder was used.

Chapter 3

Results

3.1 Fecal coliform counts

Fecal coliform bacteria are present in large numbers in the feces and intestinal tracts of humans and other warm-blooded animals, and can enter water bodies from human and animal waste. If a large number of fecal coliform bacteria (over 200 colonies/100 millilitres (ml) of water sample) are found in water, it is possible that pathogenic (disease- or illnesscausing) organisms are also present in the water. When fecal coliform counts are high there might be possibility of occurrence of waterborne gastroenteritis because these indicators are associated with waterborne illness

Fecal coliform counts in Maghbazar zone

Table 3.1.1 Fecal coliform counts in raw, boil and filtered water

MAGHBAZAR

Months	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	144.8 (n=6)	16.00 (n=3)	5.33 (n=3)	146.47	3.45	5.77
November	33.60 (n=5)	9.00 (n=2)	2.75 (n=4)	41.20	12.72	1.50
December	97.33 (n=6)	12.00 (n=2)	4.00 (n=2)	114.06	12.73	4.61
January	76.00 (n=6)	5.00 (n=2)	3.00 (n=2)	115.89	3.54	5.65
February	0.00	0.00	0.00	0.00	0.00	0.00
March	300.00 (n=3)	24.50 (n=2)	NA	173.20	4.95	0.00
April	0.00	NA	NA	0.00	0.00	0.00
Average	93.10	11.08	3.02			

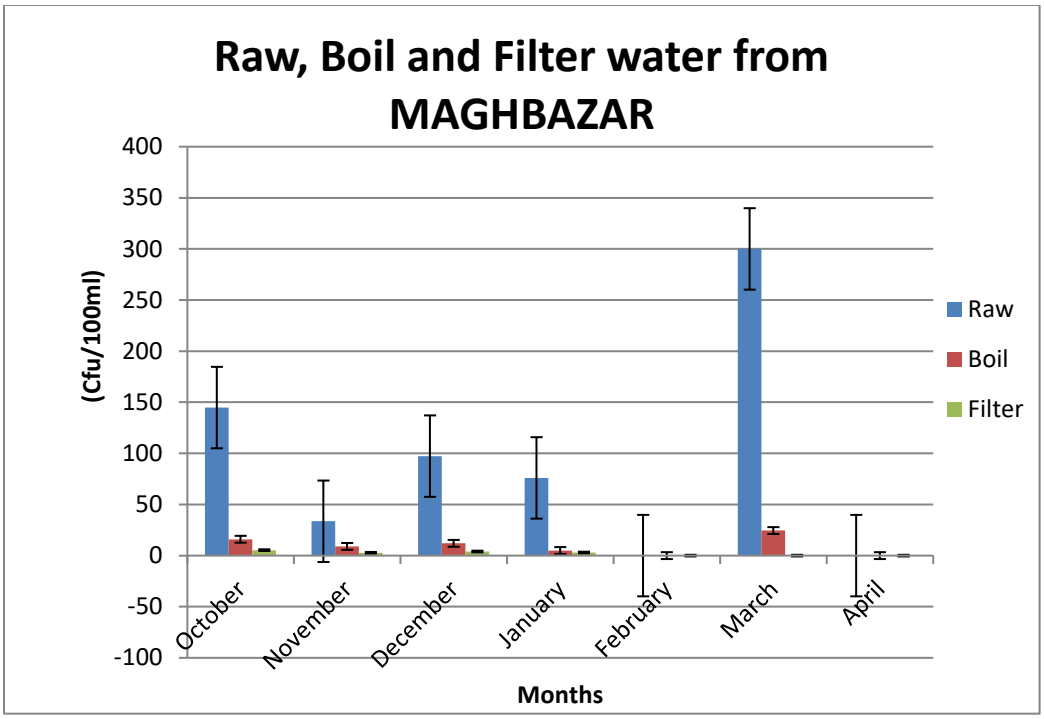

Figure: Fecal coliform counts in Raw, Boil and Filter water

Monthly variation

From Maghbazar 60 water samples were collected. The average fecal coliform count in raw water was 144.80(n=6), in boil water 16.00(n=3) and in filtered water 5.33(n=3) in the month of October . The average count for fecal coliform was 33.60(n=5) in raw water, 9.00(n=2) in boil water during the month of November . In case of filtered water the average fecal coliform count was 2.75(n=4) .In December the average fecal coliform count in raw water was 97.33(n=6), in boil water was 21.00(n=3) and in filtered water was 3.66(n=2).In January the average fecal coliform count increased to 76.00(n=6) in raw water,in boil water it decreased to 5.00(n=2) and in filtered water the fecal coliforn count was 3.00(n=2).In the month of February no sampling were done. In March, the average fecal coliform count in raw water was 300(n=2) and in boil water was 24.50(n=2). If we consider the graph we can say that the fecal coliform count in raw water during October was 122.67 ,but in November it was decreased to

33.60.The figure showed that the highest count for fecal coliform appeared in January and March and the count rose to 76.00 and 300.00 respectively.In case of Boil water samples the fecal coliform count in October was 15.00 ,which was decreased in November but again it got high in December.The result fluctuated according to the month.The count rose highest in the month of January and March.For filter water samples,the count was comparatively lower than Raw and Boil water samples.The count fluctuated between 2 to 4 which was much more lesser than the count in Raw water sample.

Standard deviation for raw, boil and filtered water:

From the graph ,we can see that the raw water samples had a higher standard deviation .In March the Standard Deviation of Raw water sample was 173.20 ,which was the highest.So,analyzing the datas we can say that the raw water samples data was more scattered than boil and filter water.

Seasonal variation:

The fecal coliform counts vary between dry and wet season .The period from October to mid November can be considered as Autumn.There could be seen light rainfall in the month of October.November to February considered as winter season.From March the summer season starts. Comparing the seasonal results it could be said that the average count for fecal coliform count was highest during summer season.

Fecal coliform counts in Badda zone

Table 3.1.2 fecal coliform counts in Badda zone

Months	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	85.00 (n=7)	11.00 (n=3)	5.00 (n=2)	45.23	10.01	5.25
November	73.00 (n=7)	16.67 (n=4)	21.00 (n=2)	40.82	14.98	14.85
December	73.85 (n=7)	15.50 (n=4)	7.50 (n=2)	48.39	9.68	2.12
January	64.28	12.00	0.00	61.25	11.34	0.00

	(n=7)	(n=2)				
April	0.00	NA	NA	0.00	NA	NA
Average	59.23	13.79	8.38			

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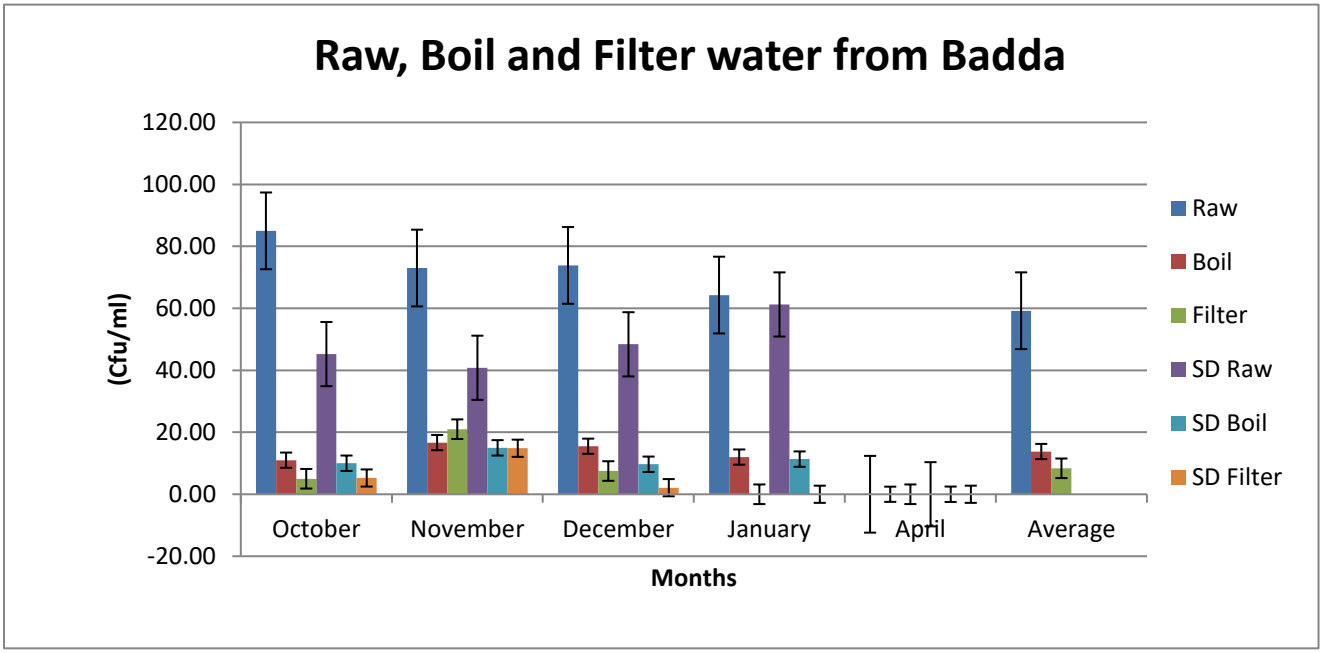

Figure: Fecal Coliform counts in Raw , Boil and Filter water

Monthly variation:

From Badda zone all together 32 samples were collected.The average count from Raw water samples in the month of October was 85.00 (n=7).In November,December and January there were no noteworthy differences in the count of Raw water samples.For Boil water the fecal coliform count varied between 11.00 to 16.67 which is much more lesser than the Raw water count.In case of Filter water the fecal coliform count in November was higher and in January the count was zero.It can be said that the highest count in Raw water appeared in October. But

it also had been seen that the count was lower in the processed water.

Standard deviation for raw, boil and filtered water:

Again from the graph it could be said that the raw water samples data is more dispersed than boiled or filtered water samples data because the Standard Deviation of Raw water samples during the month of October was highest.(SD= 45.23)

Seasonal variation:

The fecal coliform counts vary between dry and wet season .The period from October to January can be considered as Spring.There could be seen light rainfall in the month of October. From March the summer season starts. The results showed that the average count for fecal coliform count was highest during spring season.

Fecal coliform counts in Rampura zone

Table 3.1.3 **Fecal coliform counts in raw, boil and filtered water**

Rampura						
Months	**Raw Water**	**Boil Water**	**Filter Water**	**SD Raw**	**SD Boil**	**SD Filter**
October	73.60 (n=6)	17.00 (n=3)	5.00 (n=2)	28.04	15.36	4.78
November	37.83 (n=6)	5.80 (n=3)	10.50 (n=2)	14.18	5.50	8.25
December	28.94 (n=6)	10.50 (n=3)	0.00 (n=)	25.62	6.35	0.00
March	9 (n=2)	NA	3.00 (n=1)	6.36	0.00	2.12
Average	34.72	6.66	5.80			

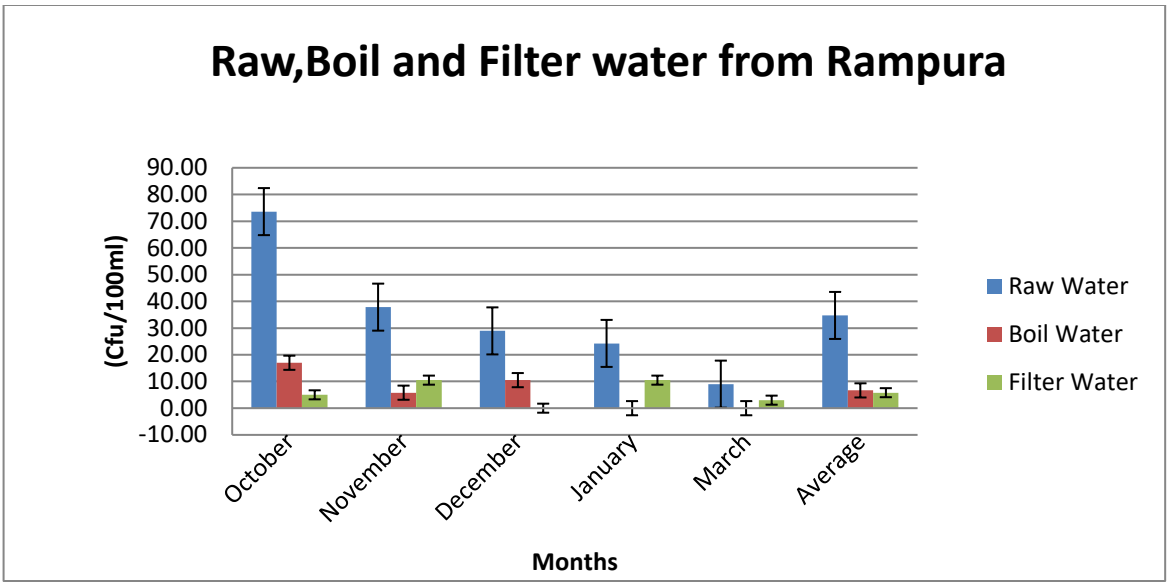

Figure: Fecal coliform counts in Raw, Boil and Filter water

Monthly variation:

A total of 29 samples were collected from Rampura zone. In October, the average count for fecal coliform in raw water was 73.60, in boil water 17.00 and in filtered water 5.00. In November the average count for fecal coliform in raw ,boil and filter was lower than October month.There were no mentionable difference in raw water count during the month of December and January.During November month the fecal coliform average count in Raw water was 37.83 ,which was decreased to 28.94 in December .For boil water the fecal coliform average count decreased to 5.80 in November but the count increased to 10.50 in December.In January the count was zero.

Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were 28.04 in October,14.18 in November,25.62 in December and 17.10 in January. From the graph we can see that, the raw water samples had a higher standard deviation it could be said that the raw water samples data is more scattered than filtered and boil water samples data.

Seasonal variation:

The fecal coliform counts vary between dry and wet season .The period from October to January can be considered as Spring.There could be seen light rainfall in the month of October. From March the summer season starts. Observing the result it could be said that the average count for fecal coliform count was the highest during spring season.

Fecal coliform counts in Bashundhara zone

Table 3.1.4 Fecal coliform counts in raw, boil and filtered water

Months	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	37.60 (n=5)	7.33 (n=3)	0.00 (n=1)	22.80	4.50	0.00
November	30.20 (n=5)	8.00 (n=3)	2.00 (n=2)	20.75	4.00	1.41
December	33.00 (n=5)	9.00 (n=3)	3.00 (n=2)	18.20	3.61	0.00
January	84.40 (n=5)	0.00 (n=1)	0.00 (n=)	67.89	0.00	0.00
March	9.00 (n=5)	NA	NA	6.36	0.00	0.00
Average	38.84	4.866	1.00			

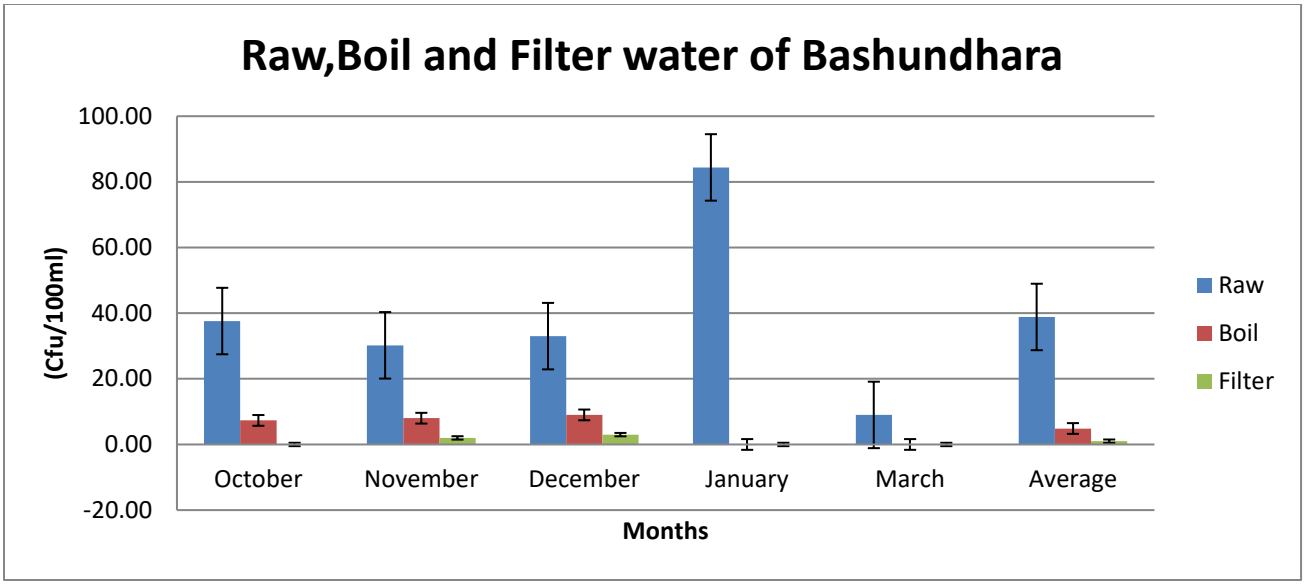

Figure: Fecal coliform counts in Raw, Boil and Filter water

Monthly variation:

From Bashundhara zone total 29 samples were collected. The average count for fecal coliform in raw water was 37.60, in boil water was 7.33 and in filtered water was 0.00 in Ooctober. In November the average count for fecal coliform in raw ,boil and filter was lower than October month.There were noticeable difference in raw water count during the month of December and January.During December month the fecal coliform average count in Raw water was 33.00 ,which was increased to 84.40 in January .For boil water the fecal coliform average count decreased to 2.00 in November but the count increased to 3.00 in December.In January the count was zero.

Standard deviation for raw, boil and filtered water:

From the graph we can see that, the raw water samples had a higher standard deviation.So it could be said that the raw water samples data is more dispersed than filtered and boil water samples data.

Seasonal variation:

The fecal coliform counts vary between dry and wet season .The period from October to January can be considered as Spring.There could be seen light rainfall in the month of

October. From March the summer season starts. So the result showed that the average count for fecal coliform count was highest during spring season.

Table 3.1.5 **Comparison between water samples of Maghbazar,Badda Bashundhara and Rampura**

Zone	Raw Water	Boil Water	Filter Water	SD Raw Water	SD Boil Water	SD Filter Water
Maghbazar	89.33 (n=6)	10.37 (n=3)	2.65 (n=3)	102.80	9.64	1.45
Badda	74.03 (n=7)	13.79 (n-4)	8.37 (n=2)	8.49	2.72	8.98
Bashundhara	38.84 (n=5)	4.86 (n=3)	1.00 (n=2)	27.73	4.48	1.04
Rampura	34.72 (n=6)	6.67 (n=3)	5.60 (n=2)	24.11	7.26	4.64

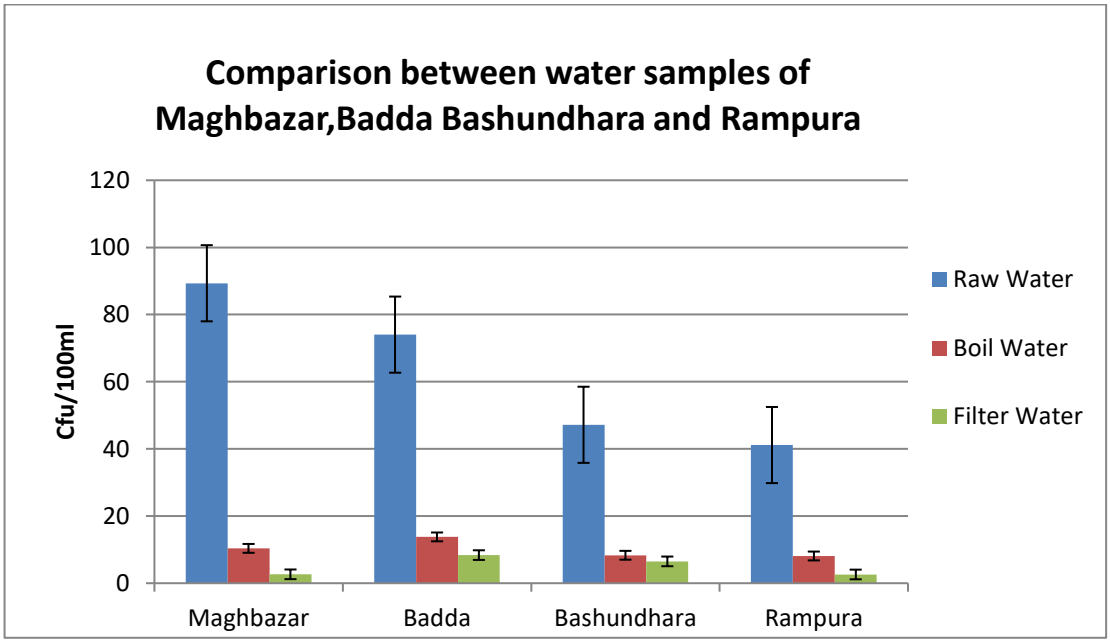

Figure: Comparison between water samples of Maghbazar,Badda Bashundhara and Rampura

We can assume that the samples represent the main water supply of Maghbazar ,Badda,Bashundhara and Rampura.From this graph,we can say that the Raw water samples from Maghbazar contained the highest number of fecal coliform count.After treatment of water the microbial load decreased to some extent.Raw water samples from Rampura and Badda contained comparatively lesser microbial count than Badda.Microbial contamination also found in treated water from this areas.The reason behind this could be unawareness of people regarding temperature or time of boiling water.Moreover microbial contamination could occur due to lack of personal hygiene , poor hanling of water or the vessels which used to keep water could be contaminated too.Furthermore, raw water samples had a higher standard deviation,so we can say that the raw water samples data is more spread than filtered and boil water samples data.

PCR result:

In this study, the extracted DNA was subjected to PCR, which amplified the invA gene, that allows for detection of *Salmonella spp.*

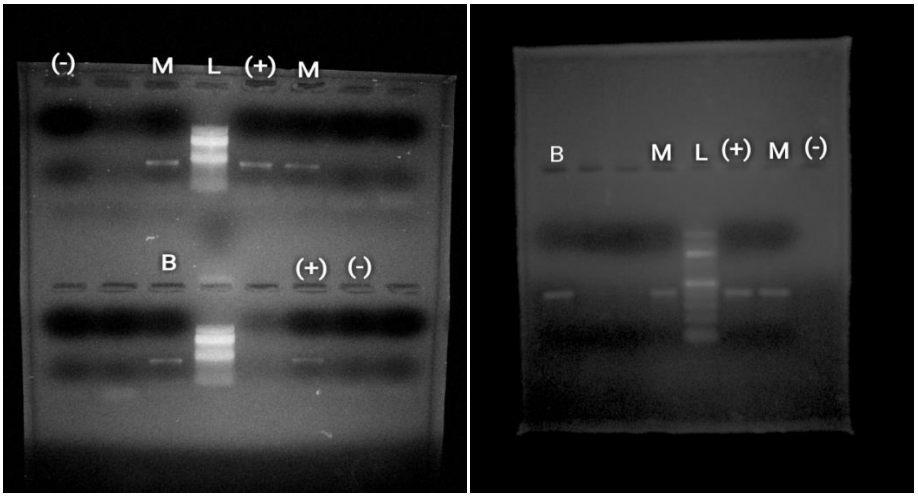

Figure 3.2.1: The PCR based results. Lanes M illustrated strains of Salmonella spp. in

Maghbazar zone and B illustrated strains of Salmonella spp. in Badda zone. L illustrated ladder which was 1500 base pair. The reference strain *Salmonella typhi* used as positive control and *Shigella dysenteriae* as negative control. The six positive bands are showed in the picture that had fecal coliform count in between 30 to 100.

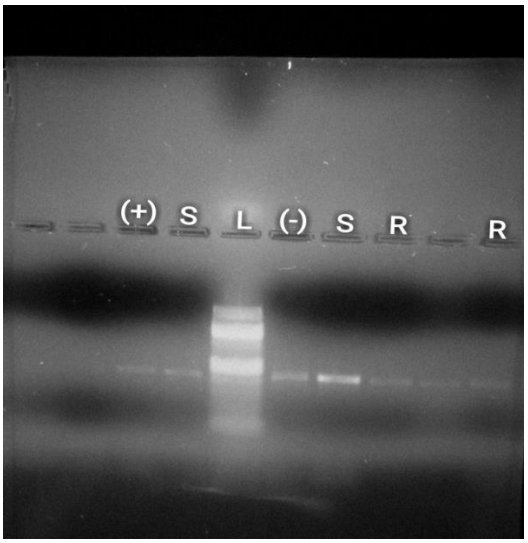

Figure 3.2.3: The PCR based results. Lanes S illustrated strains of Salmonella spp. in Shiddheswari and Shwamibagh. R illustrated strains of Salmonella spp. in Rampura zone. L represented ladder which was 1500 base pair. The reference strain *Salmonella typhi* was used as positive control and *Shigella dysenteriae* was used as negative control.The band was observed in negative control which might be the cause of contamination. The four positive bands came from the samples that had fecal coliform count above 300.

From 157 water samples.there were nine to ten samples that gave positive result for *Salmonella spp*.Among these Nine samples six samples showed positive bands from Maghbazar ,two bands showed positive results from Badda and one band showed positive results from Rampura.From the results we can say that ,the highest number of bands from these zones had been seen in the month of October and January.Though there were noticable

fluctuations in the fecal coliform count in Raw and treated water.The presence of *Salmonella spp* and fecal coliform count in water indicates that there could be possibility of bacterial contamination in the source of water or in the pipeline .Moreover microbial contamination could be occur during handling of water by a personnel.The water samples that had been examined,none of these results meet the standard quality of potable water.The presence of fecal coliform indicates that there must be a lack of efficient routine monitoring of water. Disinfection is an effective barrier to many pathogens during drinking-water treatment and should be used for surface waters and for groundwater subject to fecal contamination.Moreover it could be said that the DWASA water somehow gets contaminated after entering the distribution chain even if they are treated sufficiently (Mrityunjoy, 2011). Drinking water distribution systems play a key role in protecting public health but are also critical in supporting community development and safety.

The presence of fecal coliform count and *Salmonella spp* in treated water indicates that there might be poor hyegiene practices or the filtration machine that have been used cannot effectively remove the organisms.Previously the consumers were unaware of the dimension of pollution in the DWASA tap water otherwise they would not use it for drinking or other household purposes. The problem is rooted in Dhaka Wasa's distribution system more than the supplied water itself. The quality and safety of the water at the receiving ends depends on the quality of the source from which it is acquired,the nature of treatment given in the municipal water treatment plant and the environments in the distribution network(pipes and underground reservoirs). From this result ,it could be said that the presence of organisms in this increasing state could lead to resistance to disinfectants.The quality of DWASA water need to be under routine examination to fix this problem immediately.

Chapter 4

Discussion

Water-related diseases remain an important cause of mortality and morbidity in Bangladesh and it is suggested that intake of contaminated water acts an important mode of pathogen transmission. The disease outbreaks occur due to unsafe drinking water, inadequate sanitation and poor hygienic practices. The objective of this thesis is to evaluate the quality of water used for drinking as well as household purposes in Dhaka city, Bangladesh. The present study is conducted to identify fecal coliforms and *Salmonealla spp* from drinking water in Dhaka city. Among waterborne diseases of bacterial origin typhoid fever, bacillary dysentery and diarrhea are common in Bangladesh .The detection of pathogenic bacteria in water will help in controlling water borne infection in this region. The World Health Organization has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water or unavailability of water and at least 5 million deaths per year can be attributed to water born diseases (Karn et al, 2001).

In order to meet the ever increasing demand of safe drinking water,Dhaka Water Supply Authority (DWASA) has installed a number of deep tubewells that tap the upper aquifers. Water is one of the easiest vehicles for some of the pathogenic organisms and the contaminating water bodies may help in the outbreak of epidemic diseases ,it supports very large and genetically diverse bacterial populations that may include pathogenic strains. .The pathogenic bacteria frequently transmitted through water are those which cause infection of the intestinal tract, namely typhoid, paratyphoid diarrhea, dysentery and cholera (Pelezar and Reid, 1978). Water borne diseases constitute a major health burden in Bangladesh.

Our study reported the non-potability of drinking water samples by observing the indicator bacteria E. coli which also indicated the possible presence of other pathogenic bacteria eg *Salmonella spp*. Several factors such as environmental contamination, inadequate processing

and improper handling etc. might be responsible for contamination of drinking water . Besides, the presence drug resistance traits in the isolates might be responsible for the difficulties in eradicating the associated diseases .

Based on bacteriological tests drinking water is classified as shown in the Table 1 below

Table 4.1.1 Guidelines for determination of fecal contamination of water(Rattan 2004)

Class	Grade	Presumptive count per 100ml	*E.coli* count per 100 ml
i	Excellent	0	1
ii	Satisfactory	1-3	1-3
iii	Suspicious	4-3	3-10
iv	Unsatisfactory	10	more than 10

Most surface waters should be treated before distribution to the consumer. The Ministry of Health recommends that all supplies derived from surface water and shallow ground water sources receive disinfection as a minimum treatment. The degree of treatment needed is a function of the quality of the raw water. Protection of surface water to a degree that would eliminate health risks without treatment of the raw water is impractical in most cases. The criteria describe the raw water quality necessary for a given level of water treatment. If prevention fails to maintain raw water quality, then additional treatments or location of alternate water supplies with their added costs, become necessary. The intent of the treatment processes is to deliver water to the consumer's tap which is free of potentially harmful microorganisms, is aesthetically acceptable to the user and otherwise meets drinking water quality standards.

Table 4.1.2WHO bacteriological quality of drinking water (WHO, 1996)

Microorganisms	Guideline values
All water intended for drinking	

E.coli or thermotolerant coliform bacteria	Must not be detectable in any 100ml sample
Total coliform bacteria	Must not be detectable in any 100ml sample
Treated water entering the distribution system	
E.coli or thermotolerant coliform bacteria	Must not be detectable in any 100ml sample
Total coliform bacteria	Must not be detectable in any 100ml sample
Treated water in the distribution system	
E.coli or thermotolerant coliform bacteria	Must not be detectable in any 100ml sample
Total coliform bacteria	Must not be of samples detectable in any 100ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period

The primary objective of this research work was to assess the quality of water in Dhaka city which has been used for cooking purpose, drinking purpose and for other house hold chores . A total 157 samples were collected from different residential areas of Dhaka city including Maghbazar, Rampura, Badda, Bashundhara .For eight long months starting from october 2018 to April 2019 we have collected water from different zones of Dhaka city .Water has been collected from 27 houses from above mentioned places and from slums, as the people of slum are at high risk of disease. Among these samples 88 samples were collected from house tap,31 samples were collected from the houses who drink boiled water ,30 samples were collected from the houses who drink filtered water and 5 samples from slum. The water has been collected asceptically in a autoclaved bottle .Water samples that had been collected from the houses were under a routine examination for comparing the variation of different time period. It has also been checked how frequently people from a particular house had fallen sick from were we collected water sample for quality analysis.

In this study, 100ml of collected samples had been filtered twice; one of the filter paper placed on m-FC plate while the other was placed in 50 ml of TSB supplemented with 2.5% NaCl. m-FC plates had been inoculated at 44.5℃ and observed after 24 hours. After 12 hours of incubation of TSB 1.5 ml of it had been transferred to a 2 ml eppendrof and stock with 0.3 ml of glycerol. 1.5 ml of TSB containing bacteria had been transferred to an eppendrof which was centrifuge for 10 minutes at 14,000 rpm. The pellet was collected for DNA extraction.Molecular technique usually started with bacterial DNA extraction and purification (Ribeiro Junior et al., 2016) . PCR is so sensitive that DNA sequences present in an individual cell can be amplified. The isolation and amplification of a specific DNA sequence by PCR is faster and less technically difficult than traditional cloning methods using recombinant DNA techniques. The use of PCR substantially reduces the time and manpower required when compared with conventional methodologies. In this study, the extracted DNA was subjected to PCR, which amplified the invA gene, that allows for detection of *Salmonella* spp. (Ribeiro Junior et al., 2016)

From Maghbazar 54 samples were subjected to PCR to detect the existence of *Salmonella spp* .Among them five samples gave positive result for *Salmonella spp* ,that indicates the presence of bacterial contamination in that zone.Again from Bashundhara 35 samples were subjected to PCR for the identification of *Salmonella spp* ,but there were no positive result.Samples from Badda showed presence of *Salmonella spp* in two samples .Among 29 samples from Rampura one band showed positive result after subjecting to PCR.Analyzing this PCR result we can say that Maghbazar zone is most likely to bacterial contamination comparing to Bashundhara zone where as Rampura and Badda are least likely to bacterial contamination.

This study reveals that,the water that we use are contaminated with fecal coliform and *Salmonella spp*. The results showed that 62.79% samples of house tap water are contaminated with fecal coliform ,which exceeded the BDS standard (1240:2001) and WHO Guideline for drinking. The fecal coliform count in boil water showed 8.12% and filtered water showed 4.78% which means the findings of this study does not match with the WASA report. Dhaka Water Supply and Sewerage Authority (WASA) claims that there water is 100% drinkable but the results showing different reality.Raw water samples from Maghbazar showed the highest fecal coliform load whereas Bashundhara showed least fecal coliform count.

The bacterial load in raw water sample of Badda was also high.The number of fecal coliform load in boil water samples are comparatively lesser than raw water.The number of bacterial load significantly decreases, 62.79% to 8.12% . On the other hand microbial load in filter water is much more lower than raw water.But still it does not meet the criteria of WHO guidelines for drinking water,According to this study we can come to this conclusion that none of the sample can be said potable drinking water except those samples with zero count. There were some samples from Bashundhara which contained zero fecal count might be considered as drinkable. Finally from the graph we can say that filter water is much more safe for drinking as the bacterial load is comparatively lower than that of raw and boil water.

We can see seasonal changes in water quality,if we consider the graphs. The important water quality in the dry and wet seasons, when compared with earlier values, indicate that the degree of contamination is gradually increasing with time.In March the average fecal coliform count in raw water rose high in Maghbazar which indicates there is high possibility of bacterial contamination in water during summer . The reason behind this could be rise in temperature or

the humidity in March and April ,which is favourable for the growth of fecal coliform . There could be seen light rainfall in the month of October. From March the summer season starts.

It had been observed that the average count for fecal coliform count was highest during spring season in Badda .From Bashundhara the highest fecal coliform load had been seen in January due to the rise in temperature or fecal coliform contamination is most likely to show up during wet weather .Rainfall is the another reason for variation in growth because rain water cause sewage water to flow off and diffuse into the water supply pipeline.Rain water also washes away the domestic wastewater into the system which leads to surface water pollution and spreading of pathogens. The highest fecal coliform count had been appeared in the month of October in Rampura.Because there is a variation in temperature in October.It contains both rainy and dry weather.In first half of October month the bacterial load is high as rain water washes away the sewage water and domestic waste water into the water supply pipeline.Mostly the sampling was done during spring and winter season.If the sampling continued in summer and monsoon season,it would give a significant seasonal variation.

Every year during the dry season, people demonstrate in Dhaka's streets demanding an uninterrupted supply of clean water. The existing groundwater-based water supply system is not adequate to fulfil the water demand of the mega-city Dhaka. The results emphasize the importance of adopting appropriate routinely monitoring system in order to prevent or to diminish the chances of contamination. The bacteriological quality of pipeline and house tap water indicates that there is bacterial contamination.So the contamination could be take place in distribution system and/or domestic tanks or reservoirs. Moreover microbial contamination could be occur during handling of water by a personnel.

From our study we have understood that the city dwellers of Dhaka city are at high risk.The presence of *Salmonella spp* had been found in (10-14) % raw water samples.Among treated water samples 1% showing the presence of *Salmonella spp*.Water that we consume after treatment does not fulfil the Standard guidelines,as standard guidelines say there should be no fecal coliform in the water we consume(WHO 2008). Also we can say that after treatment of water ,the microbial load decreases to some extent.This study is a part of future project.The abstract of result has been sent to International Water Authority in Vienna. This work could be extended by checking microbial contamination which lead to biofilm formation in the vessels ,where water is stored.

References

1. Microbiological quality of sourcewaters for water supply p. 3-31 In: G. A. McFeters,edited. Drinking Water Microbiology. Springer-Verlag, New York.
2. Goel, S., Sood, R., Mazta, S.R., Bansal, P. and Gupta, A.2007. Bacteriological quality of water samples of atertiary care medical center campus in northwestern Himalayan region of India. Inter. J. Third World Med. 5 (1). DOI: 10.5580/10d
3. Nahar, A., Ahmed, M.M. and Chakraborty, A. 2011. Aquality analysis of Dhaka WASA drinking water:detection and biochemical characterization of theisolates. J. Environ. Sci. & Natural Resources. 4 (2) : 41-49
4. McFeters, G. A., Kippin, J. S. and LeChevallier, M. W.1986. Injured coliforms in drinking water. Appl.Environ. Microbiol. 51 : 1–5.
5. Mead, A.M., Helm, G., Callan, P. and Atlas, R.M. 1999. Aprospective study of drinking water quality and gastrointestinal diseases. New Eng.J. Med. 245 (9):224-248
6. Acharjee, M., Jahan, F., Rahman, F. and Noor, R. 2013.Bacterial proliferation in municipal water suppliedin Mirpur locality of Dhaka city, Bangladesh. Clean– Soil , Air, Water. DOI:1002./clen.201200618
7. Acharjee, M., Rahman, F., Beauty, S. A., Feroz, F.,Rahman, M. M. and Noor, R. 2012. Microbiologicalstudy on supply water and treated water in Dhaka city, S. J. Microbial. 1 (1) : 42-45
8. Rompre´, A., Servais, P., Baudart, J., de-Roubin, M. andLaurent, P. 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Microbiol. Methods. 49: 31-54
9. Abera, S., Zeyinudin, A., Kebede, B., Deriew, A., Ali, S. and Zemene, E. 2011. Bacteriological analysis of Geldreich, E.E. 1990.