Fecal organisms in supplied and domestically treated waters in Dhaka: Insight from Mirpur

Submitted by

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

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Dhaka, Bangladesh

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The thesis project titled "Fecal organisms in supplied and domestically treated waters in Dhaka: Insight from Mirpur" submitted by Abu Nowroze Rafin (Id-15126018) of Spring Semester, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Honours on April 25th.

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Abstract

Dhaka, the capital city of Bangladesh, has become a megacity with a population of nearly 12.5 million, which is increasing at an annual rate of over 5% (Haq, 2006).So, it is always a challenge to meet the ever increasing demand for pure drinking water of this huge population. This study aims to detect the presence of *Escherichia coli* which is a fecal coliform and also an another water borne disease causing organism which is *Salmonella* spp. Altogether,139 samples were collected from Mirpur (section-1,2,10 and 11) starting from October 2018 to April 2019.The water samples were tested . The supplied water samples collected from these four zones produced variable fecal coliform (FC) counts (cfu/100mL); 13.46% in FC count 0, 15.38% in FC count 1-5, 51.92% in FC count 6-30,19.23% in FC count 30-100 and 0% in FC count <100 from October to the beginning of February. The results also displayed overall increase in FC count during February to March month of the year which is considered as wet season which was likely to happen. Then considering the processed water which includes boil water and filter water, the results showed 65.38% in FC count 0. Then, in the detection of *Salmonella* spp, PCR had been used and bands were observed for 13 samples from the raw water samples where 5 samples came from the slums of Mirpur and korail which water samples were also detected with fecal coliform count>300 in previous procedure. This study shows the presence of *E.coli* and *Salmonella* spp in the raw water of Mirpur area and as a result people who are living in the slum are in greatest danger as they do not boil or filter their water prior to drink.

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Chapter 1

Introduction

1.1 Why water is important?

The water in our bodies is essential for life. Without water, we can't survive. Since the water in our bodies is continually being used or lost, it needs to be continually replaced, and the best fluid to replace it with is water. Water is involved in every bodily function from digestion and circulation through to the control of body temperature and the excretion of waste products. The water in our bodies is continually being used or lost from the body. Some is used or absorbed by the functions it performs and some is lost through sweat, urine and feces. . People can simply stay three days except water; however, it is viable to stay a lengthy time without nourishment. Water is particularly huge for human wellbeing, medication, farming and industry. The human body is made up for the most phase of water. Eighty-five percentage of the mind is made of water, whilst blood contains of eighty percentage water. Household water use consists of utilization of water, nourishment arrangement, washing, washing clothes and dishes, brushing teeth, watering the yard and patio nursery and so on. So, we need to check the quality of the water that if we are using safe water or not for our daily usage like drinking and other household woks.

1.2 Possibility of fecal contamination

Coliform bacteria are a natural part of the microbiology of the intestinal tract of warm blooded mammals, including man. Coliform bacteria can also be found in soil, other animals, insects, etc. The total coliform group is relatively easy to culture in the lab, and therefore, has been selected as the primary indicator bacteria for the presence of disease causing organisms. Coliform bacteria are not pathogenic (disease causing) organisms, and are only mildly infectious. For this reason these bacteria are relatively safe to work with in the laboratory. If large numbers of coliforms are found in water, there is a high probability that other pathogenic bacteria or

organisms, such as *Giardia* and *Cryptosporidium,* may be present. . Visit examination of fecal marker life forms remain the most effortless strategy for assessing the sanitation states of water. The perfect fecal pointer ought to fulfill the majority of the particular criteria, for instance, reliably nearness in the defecation, powerlessness to duplicate outside the intestinal tract, is at solid relationship with the nearness of pathogenic microorganisms, and grant straightforward research facility strategy (Savichtcheva and Okabe, 2006).Marker living beings of fecal contamination incorporate the coliform bunch all in all and particularly *Escherichia coli.*

Table 1.2 Illustration of category and color-code scheme for thermo tolerant (fecal) coliforms or *E. coli* in water supplies (World Health Organization, 1997).

Count per 100ml	**Category and color code**	**Remarks**
0	A (blue)	In conformity with WHO guidelines
0-10	B (green)	Low risk
10-100	C (yellow)	Intermediate risk
100-1000	D (orange)	High risk
>1000	E (red)	Very high risk

It is recommended that the bacteriological class plan ought to be founded on thermo tolerant (fecal) coliform microscopic organisms or *E. coli.* Gathering of point sources into classes of the sort showed up in Table 1.2 is commonly clear. By chance, be that as it may, where various

examples are taken every year, the dimensions of fecal tainting may shift broadly between dynamic examples. The clarifications behind this are regularly clear and might be identified with occasional impacts, for example, precipitation. Be that as it may, where funneled little network water supplies are being broke down and tests are taken at various concentrations in the structure, water quality may differentiate in different bits of the system at any one time. Again, the clarifications behind this may wind up clear amid the sterile assessment or if these qualifications are the after effect of cross-defilement or sullying realized by breaks in pipe work in the wake of re-sampling.

1.3 Drinking water as a vehicle of diseases

An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Wastewater discharges in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens**.** Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities.

Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water .Microbial waterborne diseases also affect developed countries. In the USA, it has been estimated that each year 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year. Among 50 maladies normal in Bangladesh, 40 of them including looseness of the bowels, diarrhea, typhoid, parasitic worm disease so on are identified with the tainted sustenance and water. Various strains of *E. coli* in drinking water are in charge of an assortment of sicknesses including looseness of the bowels, diarrhea, hemolytic uremia disorder (kidney disappointment), bladder diseases, septicemia, pneumonia, meningitis (Acharjee et al., 2011). Shiga poisons (Stx) which is known as verotoxins (Vtx), includes two noteworthy subtypes, shiga poison 1 *(Stx1*) and shiga poison 2 (*Stx2*) are delivered by a few enteric pathogens, *Shigella dysenteriae* (serotype 1 just) and enterohaemorrhagic *Escherichia coli* (EHEC). They are facultative anaerobic which can mature sugars with the creation of natural corrosive and gas. These three genera on a sort of maturation called "blended corrosive aging," anyway differentiate in different physiological qualities. More than 45,000 under-five youngsters bite the dust each year in Bangladesh from looseness of the bowels brought about by defiled water, says a report of World Wellbeing Association.

1.4 Water borne disease in Dhaka

In Dhaka, thousands of city dwellers are suffering from waterborne diseases every year by drinking unsafe water. Though, government is trying to eradicate this problem through various legislative plans and activities ,still the issue of safe water access as well as pervasiveness of water borne diseases are still very common.(Islam et al., 2001).. A number of variables may be included for contamination of drinking water. Ground water can become contaminated from

natural sources or various sorts of human exercises. Residential, civil, commercial, industrial, and agricultural activities would all be able to influence ground water quality. Contamination of tube well water seems identified with various variables, including proximity of latrines or drains to the tube wells, tube well depth or method of completion, and factors such as the practice of tube well priming may also be involved. Supplied water might be contaminated through the dissemination pipes due to leakage. It can be prescribe keeping away from releases of wastewater without treatment, mainly from septic tanks, which are extensively used in the area (Parvez, Liza, &Marzan, 2016). Diarrheal disease is a noteworthy reason of morbidity and mortality in developing countries, including Bangladesh. Among 50 diseases common in Bangladesh, 40 of them including diarrhea, dysentery, typhoid, parasitic worm infection so on are related to the contaminated water (MdShahidul, Mehadee, &Sunjukta, 2014). As children are the most at-risk group, it is important to perceive what sorts of pathogens result in their diarrhea. Studies in Dhaka, Bangladesh have demonstrated that in the stools some 75% of diarrheal children and 44% of control children have an enteric pathogen. (Ashbolt, 2004) The main organisms associated with diarrhea being rotavirus, *Cryptosporidium* parvum and the following bacterial pathogens: *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* [ETEC], enteropathogenic *E. coli* [EPEC], *Shigella* spp. and *Vibrio cholerae* O1 or O139 and to a lesser degree *Aeromonas* spp., Bacteroidesfragilis and *Clostridium difficile* (Albert et al., 1999). In any of the children in the Dhaka studies, some other potential bacterial pathogens, *Plesiomonas shigelloides, Salmonella* spp. diffusely adherent *E. coli* and enteroaggregative *E. coil*, along with the parasitic protozoa *Entamoeba histolytica* and *Giardia lamblia* were not significantly associated with diarrhoea and enteroinvasive *E. coli*, enterohemorrhagic *E. coli* [EHEC] and *Cyclospora cayetanensis* were not detected. (Ashbolt, 2004)

1.5 Selected zones of this study (Mirpur-section 1, section 2, section 10, and section 11)

Dhaka is the largest and most populated city in Bangladesh. Not only in Bangladesh but also it is one of the most populated cities in the world. The total area of Dhaka city is 300 square kilometers where 18.237 million of people are living. It is one of the most densely populated areas in the world, with a density of 23,234 people per square kilometer. Mirpur is one of the vastly populated area of Dhaka city and this area is divided into different sections such as section 1, section 2, section 10 and section 11. Mirpur has a total area of 58.66 km^2 (22.65 sq. mi) with the population of 1,074,232 and is situated in the north-east of Dhaka city.

1.6 Aim of the study

This research paper gives an overview of the microbiological quality of the raw water provided by DWASA and also the water which is purified by using different conventional techniques in the households. Eventually, this research will enlighten the people who are living in the selected zones about the quality of water, they are drinking and also how they are suffering from various waterborne diseases every year.

The main objective of this study is to determine the total load and dimensions of contaminations which leads to the quantity analysis of *E. coli* through the testing of water samples and also, the detection of *Salmonella* spp. which is the causative agent for typhoid fever. In this study, molecular techniques based on genotype of the bacteria are chosen over conventional techniques as these techniques are time consuming which include bacterial culture and serological tests.

Chapter 2

Materials and Method

2.1 Sampling sites:

A total of 139 water samples from Mirpur (section-1, 2, 10, 11)

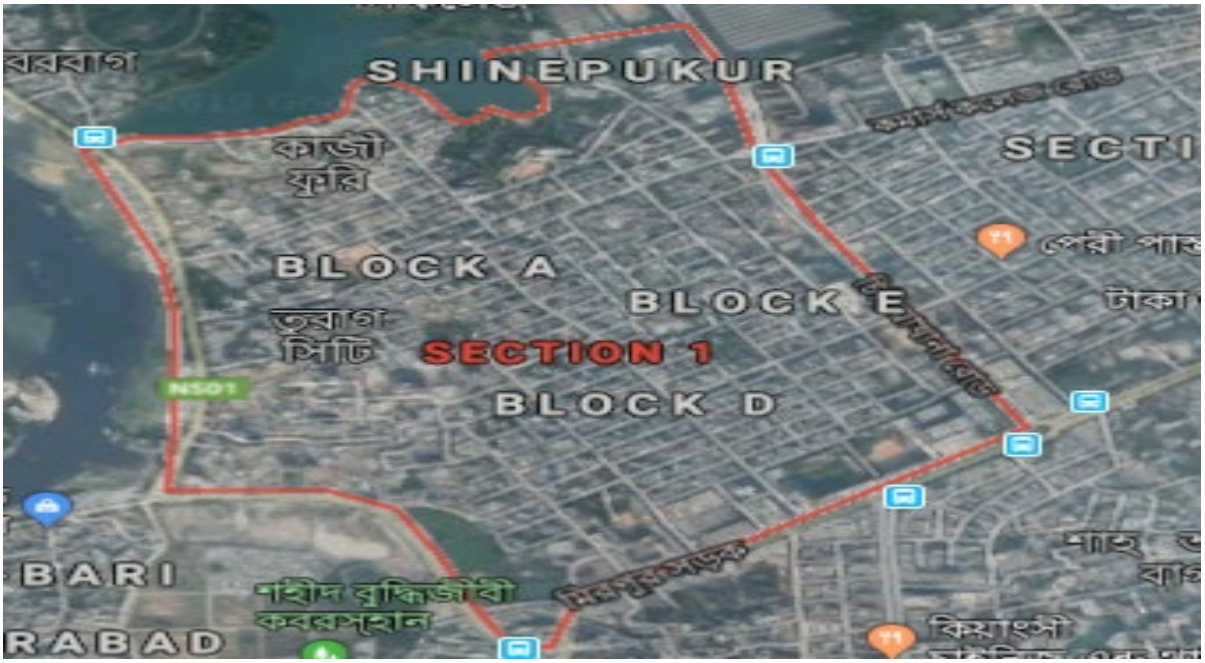


Figure 2.1.1: Satellite view of Section-1 sampling zone

Figure 2.1.1: Satellite view of Section-2 sampling zone

Figure 2.1.1: Satellite view of Section-10 sampling zone

Figure 2.1.1: Satellite view of Section-11 sampling zone

2.2 Sample collection

Water samples were monthly collected from October 2018 to April 2019. Samples were collected from different sites of Mirpur (section 1, section 2, section 10, and section 11)**.** For sample collection, the collection bottles were previously autoclaved.

2.3 Sample processing

2.3.1 Filtration

Membrane filtration method is used in this study and by using membrane filter papers,100ml of collected samples were filtered and placed on m-FC agar plates and the plates were incubated at 44.5℃ for 24 hours. After 24 hours of incubation, colonies created by fecal coliforms can seem as variable shades of blue-colored colonies on the membrane filter were observed. The colonies were confirmed by sub culturing them on EMB agar and only the colonies that had appeared as metallic green sheen are *E. coli* .Then again, the same samples were filtered in the similar manner. Membrane filter papers were placed in conical flasks containing 50ml of TSB supplemented with 2.5% NaCl and the flasks were incubated at 37◦C for 12 hours. 300µl of autoclaved glycerol were placed in autoclaved micro-centrifuge tubes. After incubation was completed, TSB were transferred to micro-centrifuge tubes and vortexed. Micro-centrifuge tubes were stored at -20◦C. 1.5ml of incubated TSB were transferred to an autoclaved micro-centrifuge tubes and centrifuged at 14,000 rpm for 10 minutes. After centrifugation the supernatants were discarded. The pellets were stored at -20℃ after the mouth of the micro-centrifuge tubes had been wrapped with paraflim for DNA extraction.

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.

2.4 Raw DNA gel run

The supernatant was exposed to gel raced to check for the nearness of DNA. Planning of introductions for PCR (stock arrangement and working arrangement):

Table 2.4 Sequences of primers used for amplification by PCR.

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

2.5 Preparation of control for PCR

Reference bacterial strains *Salmonella spp.* were streaked onto specific Medias XLD agar and brooded for 24 hours at 37ºC. After hatching, single provinces were picked and immunized in LB juices. This was hatched for 24 hours at 37ºC. After hatching LB containing micro centrifuge tubes were centrifuged for 10 minutes at 14,000 rpm. The pellets were gathered. These pellets were utilized for DNA extraction. After DNA extraction, gel electrophoresis was completed to watch if DNA were separated from these examples appropriately. After compliance PCR was performed.

2.6 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

2.7 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 include a large group of many types of bacteria that occur throughout the environment. They are common in soil and surface water. Large numbers of certain kinds of coliform bacteria can also be found in waste from humans and animals. Most types of coliform bacteria are harmless to humans, but some can cause mild illnesses and a few can lead to serious waterborne diseases. Fecal Coliform are often referred to as "indicator organisms" because they indicate the potential presence of disease-causing bacteria in water. The presence of coliform bacteria in water does not guarantee that drinking the water will cause an illness. Rather, their presence indicates that a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply. Disease-causing bacteria may use this pathway to enter the water supply.

Table 3.1 Fecal coliform counts in Mirpur (section-1) Zone

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	13(n=4)	NA	4.5(n=4)	22.79	0.0	9.00
November	29.25(n=4)	NA	3.75(n=4)	36.54	0.0	7.5
December	5.25(n=4)	NA	0.75(n=4)	6.39	0.0	1.5
January	4.75(n=4)	NA	0.5(n=4)	7.09	0.0	3
March	33.00(n=1)	NA	NA	23.33	0.0	0.0
April	80.00(n=1)	NA	NA	56.57	0.0	0.0
Average	27.54	0.0	1.58			

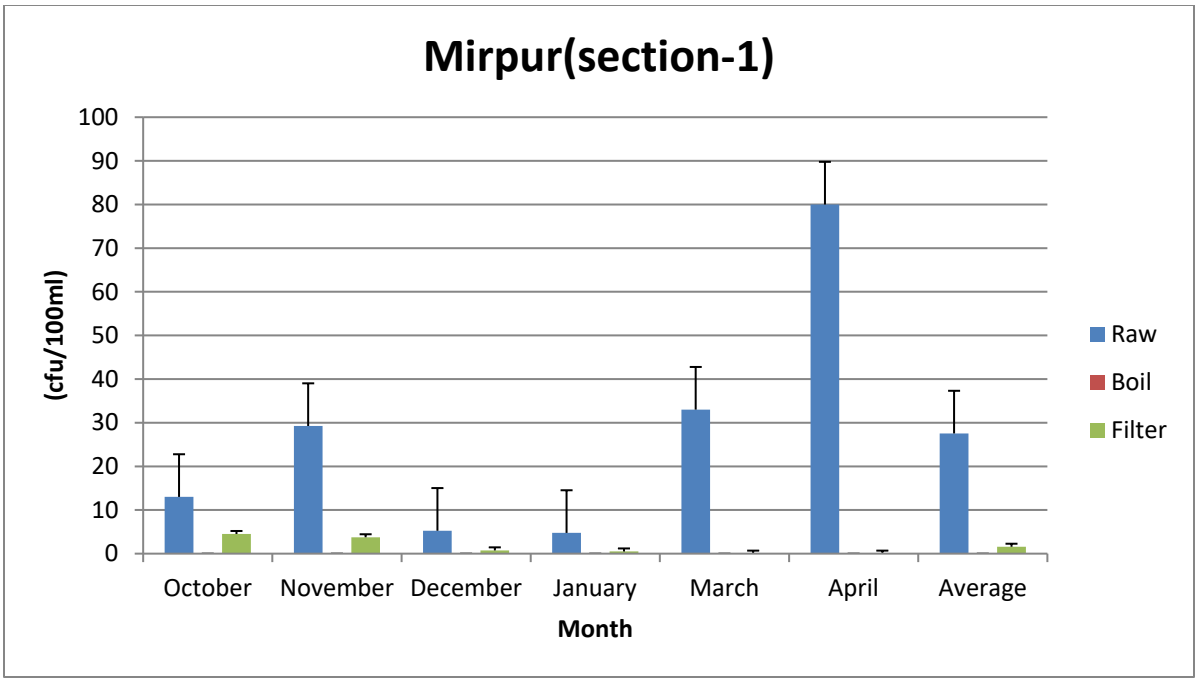


Figure 3.1: Fecal coliform counts in raw, boil and filtered water

3.1.1 Monthly variation:

From section-1, 34 water samples were collected. The average fecal coliform count in raw water was 13(n=4) and in filtered water was 4.5 (n=4) in the month of October. The average count for fecal coliform was 29.25(n=4) in raw water during the month of November. In case of filtered water the average fecal coliform count was 3.75(n=4) .In December the average fecal coliform count in raw water was 5.25(n=4) and in filtered water was 0.75(n=4).In January the average fecal coliform count decreased to 4.75(n=4) in raw water, and in filtered water the fecal coliforn count was 0.5(n=4).From October to April, no boil water samples were available. In the month of February no sampling were done. In March, the average fecal coliform count in rawwaterwas 33(n=2) and in April. The count was 80(n=2). If we consider the graph we can say that the fecal coliform count in raw water during April month was 80 which was the highest count among other months and the lowest count was 4.75, which we got in January. In terms of filtered water,

the highest fecal coliform count was 4.5 and the lowest count was 0.5 in the month of January. No filtered water samples were available from March to April. Overall, it can be said that, the fecal coliform count for raw water fluctuated from October to January month and for filtered water samples, the count was decreased gradually from October to January

3.1.2 Standard deviation for raw, boil and filtered water:

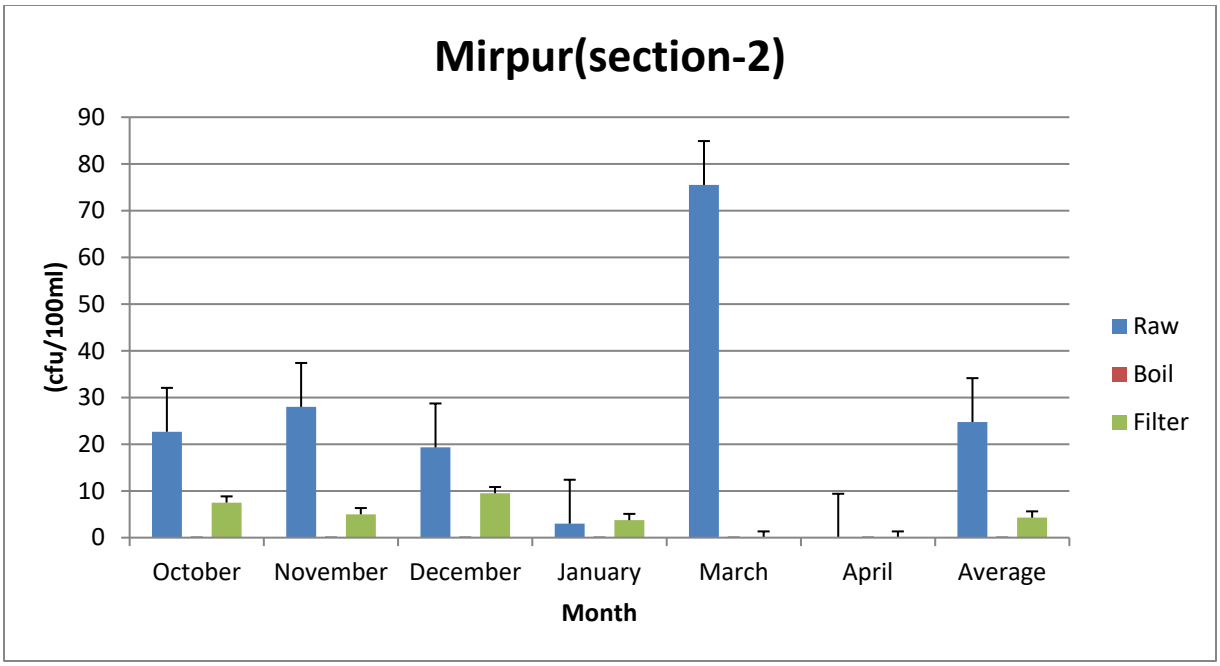
From the graph, we can see that the raw water samples had a higher standard deviation .In April, the Standard Deviation of Raw water sample was 56.67, which was the highest. So, analyzing the data we can say that the raw water samples data was more scattered than filtered water.

3.1.3 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.

Table 3.2: Fecal coliform counts in raw, boil and filtered water in section-2 Zone

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	22.67(n=3)	NA	7.5(n=3)	28.22	0.0	17.32
November	28(n=3)	NA	5.00(n=3)	21.66	0.0	11.55
December	19.33(n=3)	NA	9.5(n=3)	8.5	0.0	21.36
January	3.00(n=3)	NA	3.75(n=3)	3.61	0.0	8.66
March	75.59(n=1)	NA	NA	102.53	0.0	0.0
April	NA	NA	NA	0.0		0.0
Average	24.75	0	4.29			



3.2: Fecal coliform counts in raw, boil and filtered water in section-2 Zone

3.2 Monthly Variation:

From section-2, 26 water samples were collected. . The average fecal coliform count in raw water was 22.67(n=3) for raw water and for filtered water, the count was 7.5(n=3) during October. In October, November and December, there were no noteworthy differences in fecal coliform count for both raw and filtered water but the fecal coliform count for raw water was decreased from December to January month and the count was 3(n=3).

3.2.1 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 filtered water samples data because the Standard Deviation of Raw water samples during the month of October was highest. (SD 102.53).

3.2.2 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.

Table 3.3 Fecal coliform counts in raw, boil and filtered water in section-10.

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	17.67(n=3)	45.00(n=1)	2.00(n=3)	6.03	31.81	3.46
November	18.00(n=3)	2.00(n=1)	0.00(n=3)	11.14	1.41	0.00
December	23.33(n=3)	5.00(n=1)	1.00(n=3)	28.36	3.54	2.31
January	11.00(n=3)	8.00(n=1)	0.00(n=3)	10.54	5.66	0.00
March	8.00(n=3)	NA	NA	5.66	0.00	0.00
Average	15.6	15	0.75			

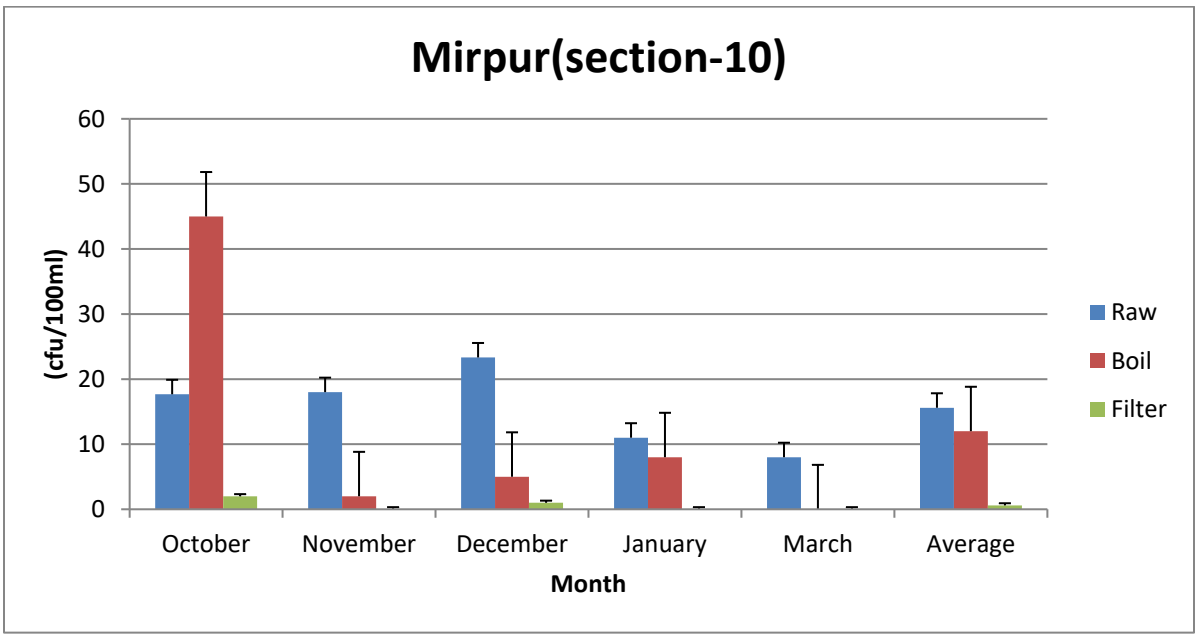


Figure 3.3 Fecal coliform counts in raw, boil and filtered water in section-10

3.3 Monthly variation:

A total of 31 samples were collected from section-10.The average fecal coliform count was 17.67(n=3) for raw water, 45.00(n=1) for boil water and for filtered water, the count was 2.00(n=3). There were no mentionable changes in average fecal coliform count for both raw water and filtered water samples from October to January. The average fecal coliform count for boil water was decreased from October to November and the count was 2.00(n=1).

3.3.1 Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were 6.03 in October,11.14 in November,28.36 in December and 10.54 in January and 5.66 in March. On the other hand ,the standard deviation for boiled water samples were 31.81 in October, 1.41 in November,3.54 in December and 5.66 in January and 5.66 in March . From the graph we can see that, if we consider the October month, the boil water sample has higher standard deviation than the raw and filtered water sample. Overall, the raw water samples had a higher standard deviation and it could be said that the raw water samples data is more scattered than filtered and boil water samples data.

3.3.2 Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during spring then summer.

Table 3.4: Fecal coliform counts in raw, boil and filtered water in section-11

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	27.67(n=3)	NA	7.00(n=3)	17.93	0.0	12.12
November	23.33(n=3)	NA	11.33(n=3)	15.28	0.0	19.63
December	19.33(n=3)	NA	2.67(n=3)	1.53	0.0	4.62
January	8.00(n=3)	NA	0.67(n=3)	4.36	0.0	1.15
Average	19.58	0.0	5.42			

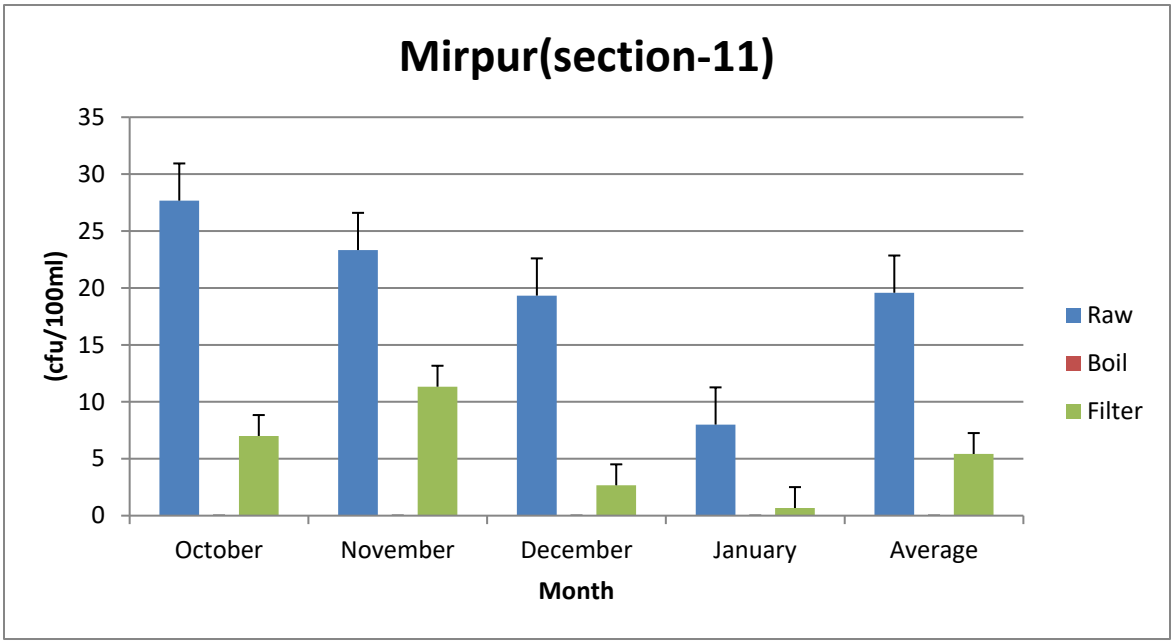


Figure 3.4: Fecal coliform counts in raw, boil and filtered water in section-11

3.4. Monthly variation

A total of 24 samples were collected from Bangshal zone. In October, the average count for fecal coliform in raw water was 27.67 and in filtered water 7. In November the average count for fecal coliform in raw water was 23.33, and in filtered water 11.33; in December the average count for fecal coliform in raw water was 19.33 and in filtered water 2.67; in January the average count for fecal coliform in raw water was 8 and in filtered water 0.67.No boiled water samples were available from October to January. Overall. It can be said that the highest fecal coliform count for both raw water and filtered water was observed in October.

3.4.1 Standard deviation for raw, boil and filtered water:

Again from the graph it could be said that the filtered water samples data is more dispersed than raw water samples data because the Standard Deviation of filtered water samples during the month of November was highest.(SD= 19.63)

3.4.2Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during spring then summer.

Table 3.5 Fecal coliform counts in raw, boil and filtered water in Slum (Mirpur)

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	336.67(n=3)	NA	NA	15.28	0.0	0.0
November	333.33(n=3)	NA	NA	37.86	0.0	0.0
December	291.67(n=3)	NA	NA	102.75	0.0	0.0
January	293.33(n=3)	NA	NA	28.87	0.0	0.0
Average	313.75(n=3)	NA	NA			

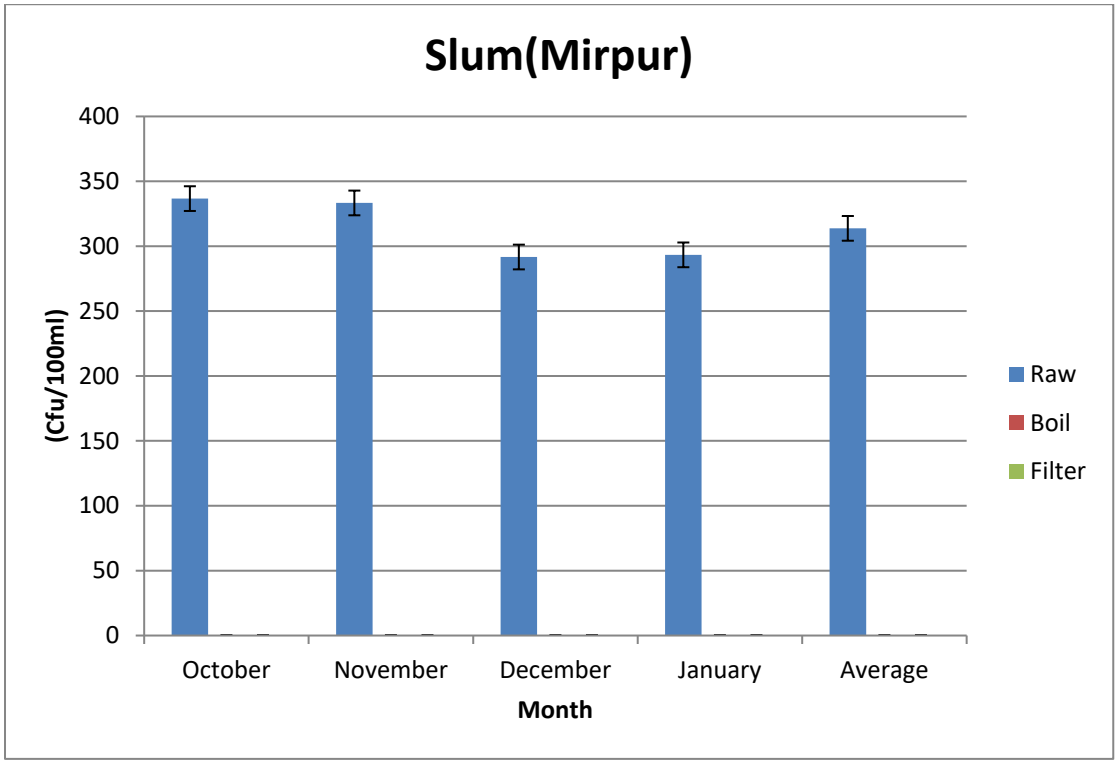


Figure 3.5 Fecal coliform counts in raw, boil and filtered water in Slum (Mirpur)

3.5 Monthly variation:

A total of 12 samples were collected from slum (Mirpur, section-1, 2).The fecal coliform count for raw water samples was 336.67 in October, 333.33 in November, and 291.67 in December and in 293.33 in January. There was no value available for boil and filtered water.

3.5.1 Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples from October to January were (336.67, 333.33, 291.67, 293.33). Only this is available so we cannot make any comparison.

3.5.2 Seasonal variation:

Spring can be considered from October to January and summer from March to April. It had been observed that the average count for fecal coliform count was highest during spring.

Table 3.6 Fecal coliform counts in raw, boil and filtered water in Hotel and Tea Stalls

Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
December	NA	NA	22.5	NA	NA	23.77
January	NA	NA	19.17	NA	NA	18.79
Average	NA	NA	20.835			

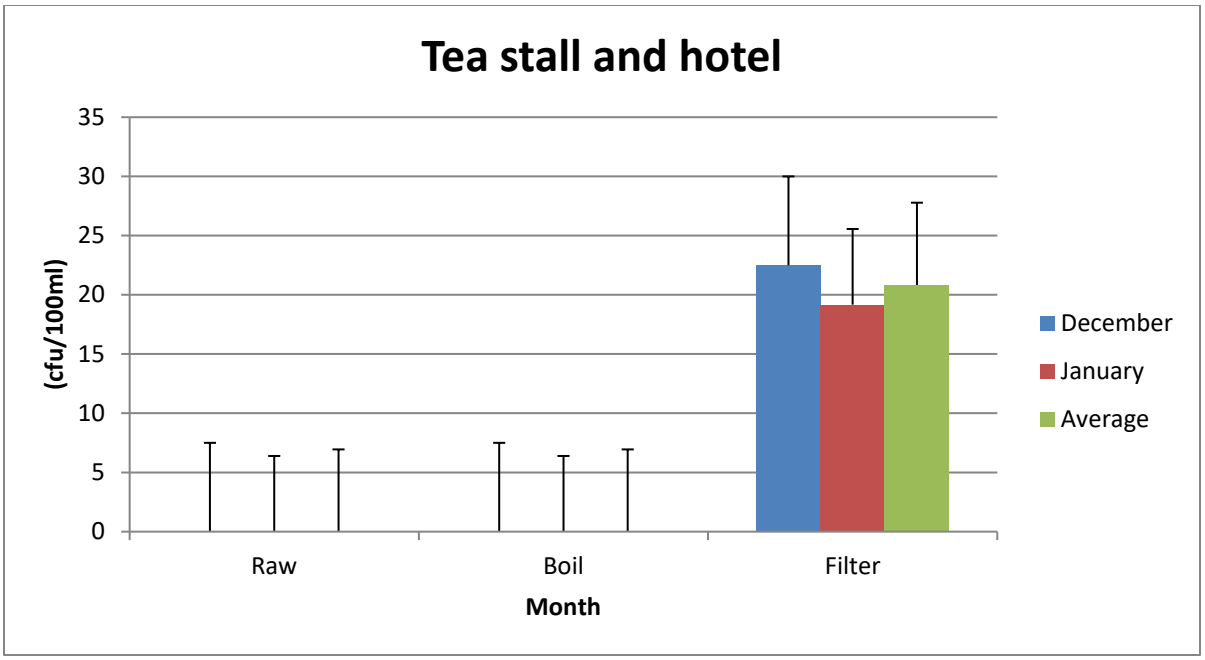


Figure 3.6 Fecal coliform counts in raw, boil and filtered water in Hotel and Tea Stalls

3.6 Monthly variation:

A total of 12 samples were collected from hotel and tea stalls. The average fecal coliform count for filtered water was 22.5 in December and 19.17 in January. No raw water and boiled water samples were available.

3.6.1 Standard deviation for raw, boil and filtered water:

Standard deviation for filtered water samples in December 23.77 and January 18.79. There is no space to make any comparison due to shortage of data.

3.6.2 Seasonal variation:

Here, the only two months have been considered January and December. So, it is not possible to make decision about seasonal variation

3.7 Comparison between water samples from section-1, 2, 10 and 11

The average fecal coliform count in raw water for sction-1 zone was 27.54, for section-2 24.75, for sectio-10 15.6 and for section-11 19.58; in boil water 0.00 for section-1, 0.00 for section-2, 15.0 for section-10, 0.00 for sectio-11; in filtered water 1.58 for section-1, 4.29 for section-2, 0.75 for section-10 and 5.42 for section-11. From the bar chart, it is clearly seen that section-1 zone contained the most fecal coliform counts followed by section-2, section-11 and section-10 for raw, boil and filtered water.

3.8 PCR result:

PCR was done using one set of primer pairs targeted for the invar gene that correctly identified *Salmonella* spp. by the size of bands products; positive bands consist of gene (403 base pair).

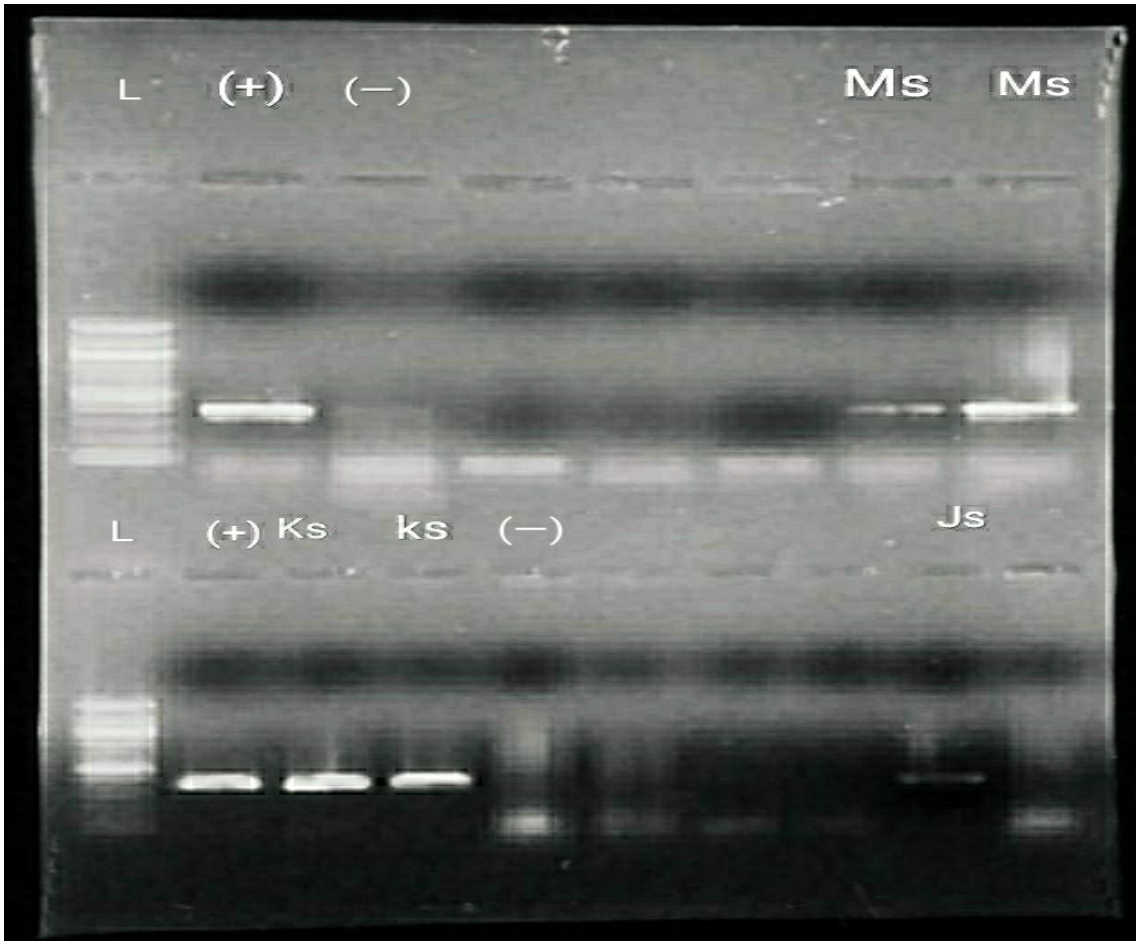


Figure 3.8.1: The PCR results. two lanes of Ms represented strains *Salmonella spp.* in Mirpur slum(section-1) zone,two Ks represented strains *Salmonella spp.* in Korail zone, Js represented strains Salmonella spp. in Janata housing(Mirpur,section-2) and L represented ladder which was 1500 base pair.The reference strain *Salmonella spp.* used as positive control and *Shigella dysenteriae*as negative control. The five positive bands came from the samples that had fecal coliform count in above 300.

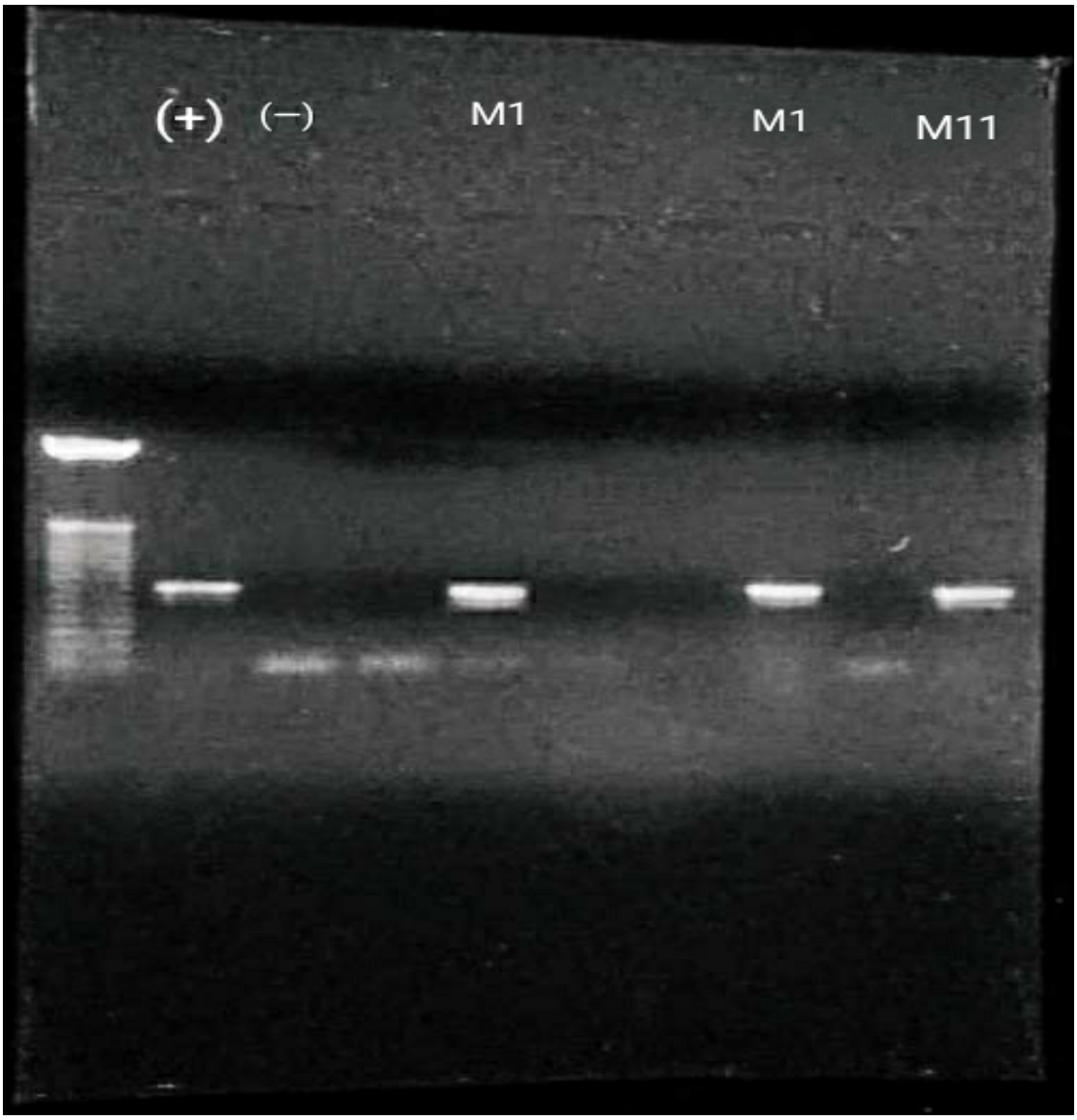


Figure 3.8.2: The PCR results. Two Lanes of M1 represented strains *Salmonella spp*. in Mirpur (section-1) and M11 represented strains *Salmonella spp*. in Mirpur (section-11) zone. The reference strain *Salmonella spp.* used as positive control. The three positive bands came from the samples that had fecal coliform count in between 1 to 5.

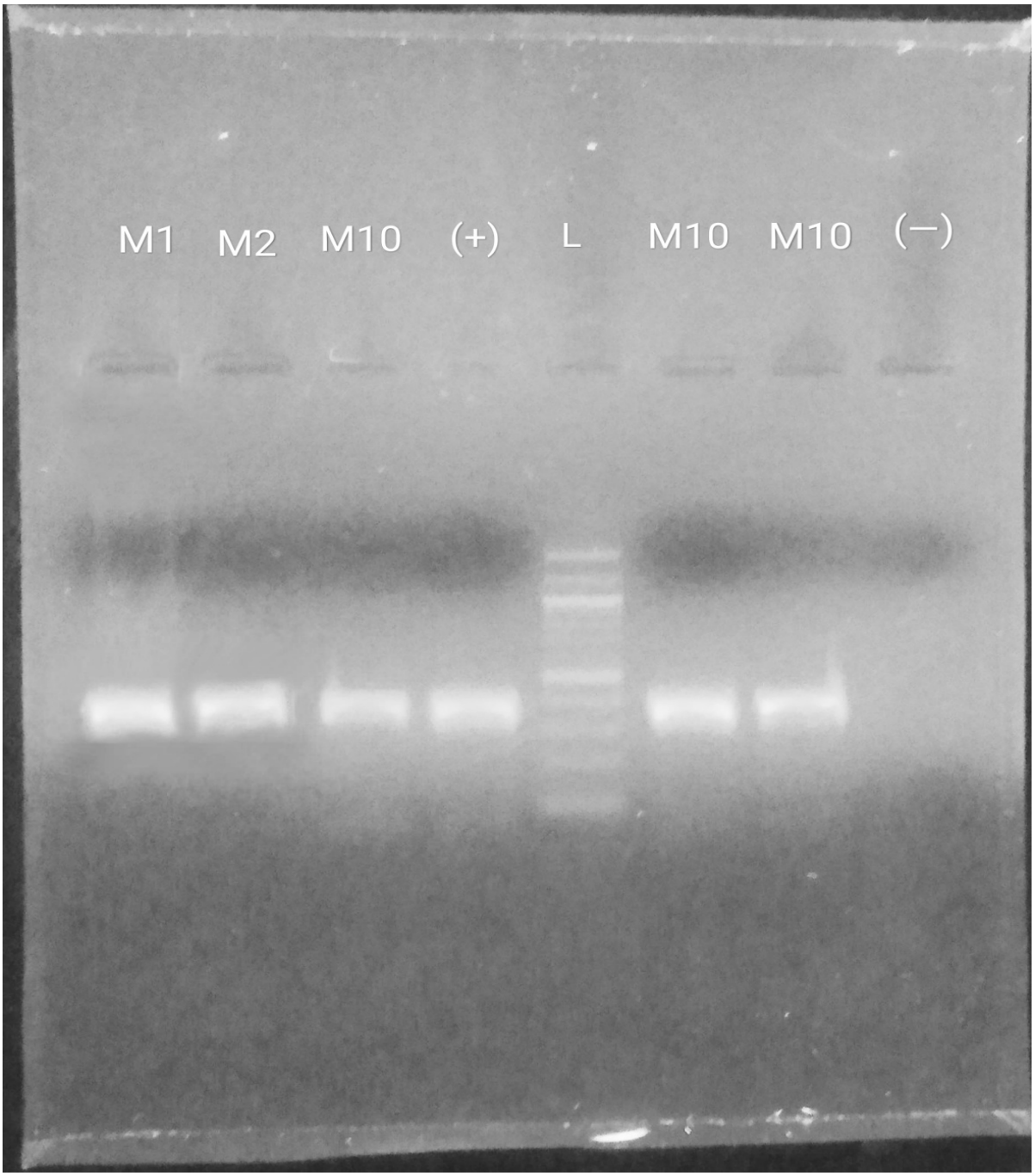


Figure3.8.3: The PCR results. Lanes M1 represented strains *Salmonella spp*. in Mirpur (section-1) zone, M2 represented strains *Salmonella spp*. in Mirpur (section-2) zone, three lanes of M10 represented strains *Salmonella spp*. in Mirpur (section-10). The five positive bands came from the samples that had fecal coliform count in between 6 to 30.

From 139 samples, thirteen samples were positive for *Salmonella spp*. Among these thirteen samples three samples with positive bands had been appeared from Mirpur (section-1) zone in November. Three samples had been observed in section-10; one in December, one in January and another in March. Three samples from slum where two were from Korail slum Zone and another one from Janata housing (section-2) had been observed in December. Another sample from slum (section-1) had been observed in October. Two samples with positive bands had been observed in January where one sample was from section-2 and another one was from section-11.Furtermore.only one sample had been observed with positive band from section-2 in March. From the results we can say that, the highest number of bands from these zones had been seen in the month of October and December. Though there were noticeable 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 hygiene 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-borne Disease is transmitted or spread through contaminated water. Pathogenic microbes and some parasitic organisms are responsible for various diseases. Such infectious pathogens survive and spread in the environment using various strategies. The main source of spread is through water. Typhoid fever and diarrhea are the most prominent issue that Dhaka city battles with on an everyday basis. City like Dhaka where 300 million of people are living required safe water for drinking and for other household work. Most of the people of this city drink water by boiling or by filtration process. The disease outbreaks occur due to unsafe drinking water, inadequate sanitation and poor hygienic practices. 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. The purpose of this thesis is to determine the quality of the water that is supplied by DWASA and the water that is used in households every day.

Clean water is a pre-requisite for reducing the spread of water-borne diseases. It is well recognized that the prevalence of water-borne diseases can be greatly reduced by provision of clean drinking water and safe disposal of feces. Better management of water resources to reduce water borne diseases and to make water bodies safe for recreational and other uses can save many lives. It also has extensive direct and indirect economic benefits, from the micro-level of households to the macro-perspective of national economies. The global importance of water, sanitation and hygiene for development, poverty reduction and health is reflected in the United Nations Millennium Declaration, in particular its eight Millennium Development Goals, in the reports of the United Nations Commission on Sustainable Development (WHO,2010).

The term “fecal coliform” has been used in water microbiology to denote coliform organisms which grow at 44 or 44.5 C and ferment lactose to produce acid and gas. In practice, some organisms with these characteristics may not be of fecal origin and the term “thermo tolerant coliform” is, therefore, more correct and is becoming more commonly used. Nevertheless, the presence of thermo tolerant coliforms nearly always indicates fecal contamination. Usually, more than 95 per cent of thermotolerant coliforms isolated from water are the gut organism Escherichia coli, the presence of which is definitive proof of fecal contamination. (Bartram and Pedley, 1996). As a result, it is often unnecessary to undertake further testing to confirm the specific presence of E. coli. One of the more important laboratory tests to determine water quality is the bacteriological test. This test indicates whether or not a given water is bacterially contaminated, and the extent of such contamination. Our study shows that the water samples came from both DWASA's supplied water and household water have the presence of fecal coliform which can be happened for several reasons 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. The maximum acceptable number of E. coli in portable drinking water should be none per 100 ml (Health Canada, 2014).

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

Tap water that is supplied by the DWASA to different households should be treated before it is provided to the consumer. DWASA claimed that they disinfect the water as a minimum treatment. However, the degree of treatment that the raw water needed for being safe and drinkable do not get most of the time. If somehow preventing the raw water from being contaminated is not getting successful, then additional treatments or location of alternate water supplies with their added costs, become necessary. The main objective of the water treatment process is to provide safe water to the consumer which is free from any type of harmful microorganisms and accepted by the consumers without any doubt.

The main objective of this research work was to determine the total load and dimensions of contaminations which leads to the quantity analysis of E. coli through the testing of water samples and also, the detectionof *Salmonella* spp. which is the causative agent for typhoid fever. A total of 139 samples were collected from different sections of Mirpur area including section-1, 2, 10 and 11. This research work has conducted for eight long months which was started from October 2018 and ended in April 2018. Among these collected water samples, 58 samples were collected from different house taps, 42 samples were collected from the filtered water sources

which people drink in those houses and 4 water samples were from one house who drink boiled water. Altogether, these water samples came from 13 houses. Only raw water samples were collected from 3 different slums as the people who were living in those slums drink only tap water and the number of samples from this section went for 12. Filtered water samples were collected from 6 different road side tea stalls and hotels and the sample number for this section went for 12. .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 where 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°C and observed after 24 hours. After 12 hours of incubation of TSB 1.5 ml of it had been transferred to a 2 ml Eppendorf and stock with 0.3 ml of glycerol. 1.5 ml of TSB containing bacteria had been transferred to an Eppendorf 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 was also carried out to detect the presence of *Salmonella* spp. in the water samples. This PCR amplifies *invar* which is a gene in *Salmonella*.139 samples were tested for PCR and among these samples, 13samples were positive for *Salmonella spp*. Among these thirteen samples three samples with positive bands had been appeared from Mirpur (section-1) zone in November. Three samples with positive bands had been observed in section-10; one in December, one in January and another in March. Three samples from slum where two were from Korail slum Zone and another one from Janata housing (section-2) had been observed with positive bands in

December. Another sample from slum (section-1) had been observed in October. Two samples with positive bands had been observed in January where one sample was from section-2 and another one was from section-11.Furtermore.only one sample had been observed with positive band from section-2 in March. From the result it could be said that none of the water samples tested was observed to be potable based on fecal coliform counts and the presence of pathogenic bacteria *Salmonella spp*. The average fecal coliform count in raw water for sction-1 zone was 27.54, for section-2 24.75, for sectio-10 15.6 and for section-11 19.58; in boil water 0.00 for section-1, 0.00 for section-2, 15.0 for section-10, 0.00 for sectio-11; in filtered water 1.58 for section-1, 4.29 for section-2, 0.75 for section-10 and 5.42 for section-11. . From the bar chart, it is clearly seen that section-1 zone contained the most fecal coliform counts followed by section-2, section-11 and section-10 for raw, boil and filtered 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 section-1 and section-2 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 favorable 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 section-10 and section-11. For section-11 zone, the highest fecal coliform count had been appeared in the month of October in 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. 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 fulfill the Standard guidelines, as standard guidelines say there should be no fecal coliform in the water we consume (Rattan 2004).

Later on there are more chances to expand this work. This work could be reached out by doing family explicit PCR and species explicit PCR ought to be finished by focusing on a harmful quality. Finally, campaigns should be arranged for general people to train them to practice some personal hygiene to prevent waterborne diseases. Hands must be kept clean by washing thoroughly with soap and water or using an alcohol-based hand sanitizer.

Chapter 5

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