

A Survey on Fecal Coliform Count and Total Coliform Count
Using Filter Drinking Water Samples Collected from Tea-Stalls
and Roadside Restaurants of Mohakhali, Dhaka.

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the
requirements for the degree of
Bachelor of Pharmacy

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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 have acknowledged all main sources of help.

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Approval

The thesis/project titled “A Survey on Fecal Coliform Count and Total Coliform Count using filtered drinking water samples collected from tea-stalls and Roadside Restaurants of Mohakhali, Dhaka” submitted by Ummay Moriom Mou of Summer, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 30.09.2019.

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Ethics Statement

The study does not involve any kind of animal trial and human trial.

Abstract

Thirteen samples of filtered drinking water were collected following the protocol of collection of water samples from roadside restaurants and tea-stalls for Fecal Coliform and Total Coliform level around Mohakhali area. Both the tests were conducted by The Central Lab of Department of Public Health and Engineering, Mohakhali, Dhaka using MFM (Membrane Filter Method). One out three samples from the fecal coliform test is found to be meeting Bangladesh and WHO standard which is zero per 100ml of sample. The level of total coliform in only two samples among ten could meet the standards. Most of the samples are found moderate to high risk of coliform contamination. Also antibiotic resistance of all the samples are conducted with Chloramphenicol, Kanamycin, Penicillin G, Ceftazidime and Cefepime which are Streptomycin antibiotics, aminoglycoside bactericidal antibiotic, Penicillin and cephalosporin antibiotic, respectively.

Dedication

Dedicated to my parents

Acknowledgement

Foremost, all the praises to Allah, Who is the source of our wisdom and, He Who has guided me in my studies, my academic career, and this very project.

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List of Acronyms

FCT	Fecal Coliform Test
TCT	Total Coliform Test
MFM	Membrane Filter Method
ARB	Antibiotic Resistant Bacteria
FIB	Fecal Indicator Bacteria

Chapter 1

Introduction

Water is the most essential substance of human body and it the specific management system that prevent dehydration of the body and ensure human survival. It is the key to mechanics of the body and without it the body cannot function properly. Water is important not only for the body but also for the industrial usage. Factories where chemicals, beverages or any sort of cosmetics are been prepared use water as their key ingredient mostly (Mishra et al., 2018). Since Bangladesh is surrounded by rivers, it is considered as one of the biggest sources of water. However, the immense pollution of water leads to limit the usability of it day by day. Due to the poor maintenance, huge amount of bacteria which are even antibiotic resistant are exposed to the environment. These bacteria are very harmful for human body and end up attacking both human and other animals livestock (Ahmed, Clegg, Williams, Baptiste, & Bennett, 2010; Crimmins & Beltrán-Sánchez, 2011; (Carlet et al., 2012). The present scenario of the country' health care system is highly affected by this type of antibiotic resistant bacteria and the number is increasing enormously. The death rate is increasing so fast yet one of the biggest reasons behind this is the increasing number of bacteria surrounding the community. Water portability of bacteria are been chosen as an indicator from the Enterobacteriaceae family (Fewtrell, Bartram, Ashbolt, Grabow, & Snozzi, 2001); (Behera & Mishra, n.d.-b); (Behera & Mishra, n.d.-a). Bacteria from this family are being able to reproduce in the stomach of mammals as they have warm blood, help to indicate the presence of any pathogen and detected in the feces. These bacteria are very resistant to the drug genes and are being able to modify themselves into other pathogens in a short time. Thus, they are causing hazard to the health. So, monitoring of fecal coliform and total coliform level in the water are very crucial to maintain healthiness and reducing the hazards (Mishra et al., 2018).

1.1 Arising of waterborne diseases

Contaminated or dirty water leads to waterborne diseases. Every year about 3.4 million deaths are caused due to water related diseases. Contaminated water increases the growth of diarrheal diseases including Cholera and other life threatening diseases like Guinea worm disease, dysentery, typhoid and other serious diseases which contains pathogens and affect the human body. Because of diarrhea, about 4% deaths and 5% health loss to disability occurs every year and mostly the gastrointestinal infections kill around 2.2 million people every year globally. In our country, about two out of five households which is almost 38.3 percent of the population drink water that are sourced from contaminated water sources which are disease-causing bacteria and viruses (White, Bradley, & White, 2002). Poor hygiene practices at households' increases the consumption of contaminated water and the number jumps to 98 million. Conditions of the hilly terrains, islands, coastal regions, urban slums and wetlands are worse and there is no reliable source of accessing to safe water. Moreover children's below age group of 5 are unable to meet the nutritional requirements. Mostly children are under weight and lack of nutrient in their body and give access to the enteropathogens to enter inside the body (Balows & American Society for Microbiology., 1991). A study conducted in Bangladesh shows 40% of the children' complimentary foods are being contaminated by Escherichia Coli. Level of fecal coliform and total coliform are highly influenced by the quality of drinking water. The side effects of each substance are not easy to be identifying separately and are linked by fecal oral-pathway which is the point of context (Bain et al., 2014).

1.2 Access to Safe water sources

The basic foundation for all human includes the right to have safe water for their survival. 97% of the population have the access to improved water sources in 2013 which is a significant progress for Bangladesh in terms of universal access to safe water (White,

Bradley, & White, 2002); (Guidelines for Drinking-water Quality Forth Edition WHO Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4th ed, 2011). Still the drinking water being safe is at a low rate of 34.6 percent. Despite those progress has been measured yet 19.4 million people of Bangladesh are still drinking water which is above the level of national health standards. According to the 2013 MICS survey, more than 41% people drink water with faecal contamination which rises to 61.7% on consumption point. The survey also indicates that the manner of microbial contamination is worsening the water as it is been transported from sources to the households. Due to poor hygiene practices in households, the number of contamination is rising enormously. UNICEF has found evidence that significantly shows that disparity in water quality in different regions of Bangladesh are still present. For instance, Rangpur division has about 71.8% water that is E.coli free whereas Sylhet division can only access the same water at 31.6% rate. Also drinking water sources are being contaminated very frequently in times of disasters like floods, landslides and cyclones. The barriers to have safe drinking water alongside sanitation have significant impact in the health, education, nutrition, protection and other outcomes which affects the children and adolescents (Guidelines for Drinking-water Quality FOURTH EDITION WHO Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4th ed, 2011); (Report JMP Technical Task Force Meeting on Monitoring Drinking-water Quality, n.d.).

1.3 Standards for Safe drinking water

World Health Organization (WHO) has produced international norms for water quality and human consumption of water to ensure sound health in the form of guidelines which are used as the base of regulation and standard setting worldwide. Those guidelines are known as “Guidelines for drinking-water quality” (GDWQ) which promotes the protection for the community as public health as advocating the development of local relevant standards and regulations (on health based targets) by adopting some preventive risk management settings

that can cover catchment to consumers (Water Safety plans) and for independent surveillance as ensuring the Water safety plans for being implemented and that are effective enough for the national standards meet. As the guideline directs, any 100 ml water sample must be E.coli none detectable. The process of quantification is much easier while using E.coli or any type of thermo tolerant coliform whereas latest enzymatic processes are considered as simpler and less costly. The GDWQ has also recommended that this process can be used as an alternative for the identification of faecal contamination in drinking water (Bain et al., 2014). Sanitary inspection is one the most widely used and effective way of identifying the hazards and helps in the risk management system. The sanitary inspection results can vary depending on time (seasonal mainly) and on area (Lloyd & Bartram, 1991), (Clasen & Bastable, 2003); (WHO, 2009). Whereas use of improved indicators can work as a simplified way for sanitary inspection which can determine the level of contamination in the water sample. Moreover, WHO GDWQ have suggested that use of risk classification for the highlighting of interventions as this can detect the higher level of fecal coliform presence by the higher level indicators (The Coliform Index and Waterborne Disease, n.d.). On the basis of counting indicator organisms in every 100ml it is considered in three different types of risk classification which are (1)Very low risk (1-10 coliform/100 ml), 2. Low risk (10-100 coliform/100ml), 3. Medium risk (100 coliform/100ml), 4. High risk or very risk (more than 100 coliform/100ml) (Clasen & Bastable, 2003), (WHO, 1998). Though this classification is not considered as perfect to detect the actual number of coliform as it varies on the basis of time (seasonal) and area. The standardized forms include assessment of sanitary risk with measures taken by sanitary score.

1.4 Coliform Contamination

Coliform bacteria are the organisms that are found in the feces of warm blooded mammals and present in the environment. Normally they are considered as not causing any illness.

However, presence of coliform bacteria indicates that the water must contain disease causing organisms or pathogens (Pruden, Pei, Storteboom, & Carlson, 2006); (Diehl & LaPara, 2010); (Dodd, 2012); (Chen et al., 2015). These pathogens can contaminate the water system from the waste of mammals and also from poor maintenance of sewage and pipelines. To identify all the pathogen through testing is a very complex, expensive and time consuming process so it is very convenient to test for the coliform bacteria. After test, if any presence of coliform bacteria is found then the water system operator should work for finding the source of contamination and ensure safety of the drinking water. Coliform bacteria are classified in three different groups which include total coliform, fecal coliform and E.coli respectively. All three of these coliform are considered as indicators of drinking water quality.

Total coliform contains a large number of different kinds of bacteria. These are commonly found in the environment and are generally considered as harmless. If any source of water is found containing total coliform then it is considered that the source is probably contaminated environmentally. However, entering of environmental contamination can act as a way for the pathogen to enter into the source so it is important to find the source of contamination and resolve it as soon as possible.

They are present inside the intestines and feces of mammals in a great quantity. Fecal coliform is the type of total coliform that mostly exist in feces of mammal. Any sample containing fecal coliform indicates that there must be a recent fecal contamination and can result in great risk of pathogenic organisms presence.

When any presence of coliform bacteria is observed in the water system, it should be investigated properly to find the source of contamination and how it got into the water. Normally collecting additional or “repeat” water samples helps yet sometimes entire system has to be inspected (Scully, Hartman, Rule, & Leblanc, 1996); (Jaglic et al., 2012);(Cherchi

& Gu, 2011). If repeat samples are collected and tested then it helps to determine whether the problem actually exists in the system. If repeat testing of the samples give results of presence of coliform bacteria then the initial findings are considered as confirmed contamination.

1.5 Fluctuation of Fecal Coliform (FC) Count

One of the major issues of fluctuation of fecal coliform count includes seasonal change. It can be divided on the basis of humidity, temperature and also on different seasons like summer, winter, and autumn. It is been considered that microorganisms are more vulnerable during rainy season as they have more exposure to the environment (Onda et al., 2013). They can grow widely and their mobility is at the peak in wet season. Though most surveys on drinking water are conducted in dry or winter season for the flexibility of movement. If the seasonal trends are considered than there will be inaccuracy in the results of contamination level and thus lead to more inaccurate results. Following seasonal patterns are a great parameter for water quality determination which is a very common trend in previous studies (Ouyang, Nkedi-Kizza, Wu, Shinde, & Huang, 2006). The guidelines can be polished considering the sample collection on the basis of seasonal change which will eventually help in enhancing the area of sample collection and provide more accurate results. Though no review on general seasonal pattern on fecal contamination and total contamination from the source of improved drinking water sources are conducted in developing countries (Kostyla et al., 2015). In case of developed countries studies have significant sampling in different season which includes both improved and unimproved drinking water sources (R. C. Wright, 1986).

1.6 Fluctuation of total coliform (TC) count

Bacterial contamination can be introduced to water from many sources including natural, man-made or by its own activities. Feces from warm blooded animals including human

contains disease- producing microbes that consist of pathogens, viruses even intestinal parasites (Prüss-Ustün et al., 2014); (Sarkar, Sivarathinaswamy, et al., 2013); (Sarkar, Ajjampur, et al., 2013). Though total coliform indicates bacteria those are not harmful for human body yet help by acting as an indicator that identifies presence of other harmful bacteria. Though fluctuation of total coliform count are significant as sewerage areas are increasing in recent years and the increase of bacterial discharge have resulted despite the use of advanced waste treatment methods (Achieving water security for Asia Assian Outlook Development 2007, 2007); (Alarcon Falconi et al., 2017), (Kumpel & Nelson, 2016). The disposal of untreated and non-disinfected human wastes is one of the primary causes of contamination. However, increasing contact with the surface water has resulted in more exposure of humans directly to this bacterially contaminated water including drinking water (Eshcol, Mahapatra, & Keshapagu, 2009), (Levy, Nelson, Hubbard, & Eisenberg, 2008), (Mintz E, Reiff F, & Tauxe R, n.d.). According to WHO GDWQ standards, there should be no coliform present in every 100ml of sample and studies have found that even if high measures are taken in account to consider the safety of the water that are used for direct consumption are still not free of coliform (Bhunia, Ramakrishnan, Hutin, & Gupte, n.d.); (Alarcon Falconi et al., 2017); (J. Wright, Gundry, & Conroy, 2004).

1.7 Effects of Fecal Coliform on human

Among the most common pollutant in streams and rivers are the fecal coliform bacteria. According to report, about 37% of surveyed river water, 31% of lakes and 23% of estuarine water has some level of contamination (Bhunia et al., n.d.); (Achieving water security for Asia A sian O utlook D evelopment 2007, 2007); (Alarcon Falconi et al., 2017), (Kumpel & Nelson, 2016). The most possible sources of elevated level of coliform counts include sewage discharges that are from municipal treatment plants and septic tanks, runoff from pastures, storm water overflows and range lands. Also waterways are continued to have higher levels

of unhealthy bacteria because of the human wastes and natural contaminants. Studies conducted during different seasons on water contamination have showed that mostly in rainy season the levels of bacterial contamination are found to be increased. Mainly water pollution due to fecal contamination is been a serious issue as a potential for contracting diseases from pathogens which are disease causing organisms. Perhaps, concentrations of the pathogens from fecal contamination are smaller in number than other possible pathogens (Lyimo, Buza, Subbiah, Smith, & Call, 2016); (Bergeron et al., 2015) and DWTPs (Bai et al., 2015); (Jiang et al., 2013); (Guo et al., 2014). As a result, it is not possible to test for pathogens in all the water samples being collected. In contrast, pathogens are tested with indirect evidence by testing the samples for an “indicator” organism such as coliform bacteria (Bhunias, Ramakrishnan, Hutin, & Gupte, n.d.); (Alarcon Falconi et al., 2017); (J. Wright, Gundry, & Conroy, 2004). Coliform bacteria are comparatively very easy to determine their presence as they are usually in larger numbers than harmful pathogens and they respond to the environment through water-waste treatment.

1.8 Importance of testing Total Coliform (TC) Count

The presence of bacteria and other disease causing organisms also known as pathogen is a major concern while considering the safety of the drinking water. Pathogenic microorganisms are responsible for intestinal infections, hepatitis, dysentery, typhoid fever, cholera and other sicknesses. Waste products of the mammals are primary sources of bacterial contamination in water. The other sources include feedlots, pastures, dog runs and other land areas where animal wastes are found deposited (Allen et al., 2010); (Gaze et al., 2011); (Wellington et al., 2013). Additional sources may include seepage or discharge from septic tanks, sewage treatment facilities and other natural soil or plant bacteria. These bacteria can enter into wells or other open surface of water that are not water tight casing or have caps on them. As studies suggest, every water sources should be tested at least four times per year. EPA has

established standards for drinking water and those are categorized in two groups known as 1. Primary standards and (2) Secondary standards. Among them primary standards are based on health considerations and designed to protect human from three types of toxic pollutants which include pathogens, radioactive elements and toxic chemicals. Contamination of bacteria falls into the pathogen category. As testing for all individual pathogen is comparatively impractical and costly so EPA has designed total coliform bacterial count as a standard to determine the safety of water. This test helps to estimate the increase or decrease in number of pathogenic bacteria by monitoring the coliform bacteria. No specific sanitary standard significance or health standards are been indicated for non-pathogenic non coliform bacteria other than a total heterotrophic bacterial count or standard plate count of <500 Colonies per ml. When a water sample is tested and reported as not meeting the EPA bacteriological standard of zero it is considered as dangerous to consume. Sometimes excessive number of other bacteria can interfere in a sample while counting the coliform types. All these samples are classified as “too numerous to count” or “confluent growth” sources (Lyimo, Buza, Subbiah, Smith, & Call, 2016); (Bergeron et al., 2015); (Bai et al., 2015); (Jiang et al., 2013); (Guo et al., 2014).

1.9 Antibiotic Resistance

Antibiotic resistance has now become one the greatest threat for the global health as well as food security. Resistance to antibiotics sometimes occur naturally but mostly it happens due to misuse of antibiotics in human and animal (Pruden, Pei, Storteboom, & Carlson, 2006); (Diehl & LaPara, 2010); (Dodd, 2012); (Chen et al., 2015). Antibiotics are the medicine used to treat and prevent any sort of bacterial infections. Antibiotic resistance occurs when the bacteria changes its response towards the medicine and do not work as expected. Bacteria that are resistant are harder to treat then those of which are non-resistant. To solve the problem, the world needs to change the way of prescribing and using antibiotics urgently.

The presence of new medicine will not be at good if the behavior is not changed. Behavior change includes action to reduce the spread of infections by vaccination, hand washing, practicing safer health and good food hygiene. Normally in countries which do not have a standard treatment guidelines are often over prescribe antibiotics by the health workers and veterinarians and the public end up over using it. A UK commissioned report (Allen et al., 2010); (Gaze et al., 2011); (Wellington et al., 2013) have fears of 10 million deaths because of antimicrobial resistance by 2050. Antibiotic resistance has become a worldwide problem and Bangladesh is one of the major contributor to that owing its poor healthcare standards and misuse or overuse of antibiotics at a high level. This threatens the effective prevention and treatment of the increasing range of infectious diseases caused by bacteria, viruses, parasites and even fungi. One of the reason behind antibiotic resistance is the research on antibiotics are highly expensive and time consuming so the very few scopes of antibiotics research of new high class antibiotics remain out of reach (Khan, Beattie, & Knapp, 2016).

1.10 Objective

People around both the cities and towns are very much likely to have roadside restaurant foods and the number is huge. Perhaps the possibility of having clean and safe drinking water at least is still a great challenge. The issue of microbial contamination in drinking water is still an alarming risk and it has its wild effects on human health. Though people are now very much aware of the fact about waterborne diseases and they are very much cautious about their choices in terms of safe water as many incidents of municipal water supply is been reported. The reasons behind this type of contamination include low maintenance of both transportation and pipelines and also leakage in those pipelines. A few companies have come forward for the supply of water bottles that claimed to have filtered water yet it is costly for normal people. An alternative for this solution was supplying water at a low cost in big sealed containers. These containers are easy to use as it needs to be attached to the dispensing

machine and no wiring or heavy maintenance is required. Consumers can have the water in small glasses directly from that dispenser. Now that drinking water from this type of dispenser are very much common and have gained popularity in recent times, it has become an important part to check whether those water are safe enough to drink directly and to ensure that the water are microbial contamination free. The testing of microbial contamination in these containers of drinking water are being tested by Moniruzzaman et al. in that study count of TC, FC and HPC has been obtained. A monthly newspaper known as “BRACU Express” published by the students of Brac University in November 2018 about restaurants and hotels that are budget friendly for students around Brac University Mohakhali area. Almost every student of Brac University has visited those restaurants at some point of their journey. This data gave a serious need of testing the microbial contamination in those restaurants. Two students from the department of pharmacy have already conducted a survey on the physical properties and FC count of some samples around the area and their results are unexpected. Following those results it was a necessity to test the drinking water quality of the restaurants around the campus area. The goal of this survey is to count the fecal coliform count of the particular samples which was found very high in their data and total coliform count of 10 drinking water samples collected from different restaurants and tea stalls around Mohakhali, Dhaka.

Chapter 2

Methodology

2.1 Site Location and Area

Brac university premise which is situated at Mohakhali area in Dhaka city is a well-known commercial area that includes many institutions like Banks, private firms, hospitals and other offices of different organizations. About hundreds of hotels, roadside restaurants and small carts supplies food and beverages in the area.

2.2 Sampling Site and Sources

Thirteen filtered drinking water samples from different restaurants, hotels and tea-stalls around Mohakhali area were collected to count the fecal coliform and total coliform level. Among hundreds of restaurant busiest one were selected for sample source. All the samples were collected from 26.08.2019 to 01.09.2019. Two sources are used to collect the samples which include the WASA and filtered drinking water from private companies supplied in blue jar containers.



Figure 1: Map representing restaurants and tea-stalls around Mohakhali using Arc GIS

Figure 1 shows the location of restaurants and tea-stalls around Mohakhali area. The students of Brac University are mainly exposed to these food restaurants and almost every student has their daily meal in these restaurants. The location coordinates are found by using ArcGIS (Version 10.5) which is an Architecture Geographic Information System that is commonly used by the urban planners and geographers. It shows the maps and geographic information of lands.

2.4 Tests for Antibiotic Resistance

Chloramphenicol is the antibiotic of Streptomycin group which is used in the treatment of a number of bacterial infections. It can be used as ointment for eyes to treat conjunctivitis, to treat meningitis, cholera, plague and also typhoid fever. It works by stopping the growth of bacteria. It only treats bacterial eye infections rather than other eye infections.

Kanamycin is an antibiotic from the group of aminoglycosides which fight bacteria inside human body. It works by inhibiting the synthesis of proteins in susceptible microorganisms. It acts as bactericidal in vitro against Gram-negative and certain types of bacteria of gram-positive. Mostly this classes of antibiotics are ineffective against salmonella and shigella species patients.

Penicillin G, in the group of penicillin antibiotic is considered as fast-acting antibiotics which fight bacteria in human body. It can be used in the treatment of severe infections including staph and strep infections, diphtheria, gonorrhoea, syphilis and also meningitis. It can also treat ear infections in patients. Though it can have some side effects if not used properly like-injection site reactions, nausea, upset stomach, diarrhea and overactive reflexes.

Ceftazidime is a cephalosporin group antibiotic that is been used to treat a wide range of bacterial infections. It works by stopping the growth of bacterial organisms. It can be used to treat severe to life threatening forms of bacteria. It can be administered by intravenous injection or infusion or else by deep intramuscular infusion.

Cefepime also belongs to the cephalosporin antibiotic group that can be used in the treatment of pneumonia, skin, kidney and UTI (Urinary Tract Infection). It is broad spectrum antibiotic which can work to inhibit a wide variety of bacteria.

If antibiotics are taken when not necessary to use them can result in resistant to that particular type of antibiotic. Previously a survey study conducted by students of Brac university have

found some samples around the Mohakhali area are resistant to some narrow spectrum antibiotics so broad spectrum antibiotics are being test on the samples to identify any presence of resistance towards this antibiotics. But no resistance was found in the samples. The test was performed in the Microbiology Laboratory of Department of Pharmacy in Brac University following standard protocol.

2.5 Methodology for Antibiotic Resistance Test

Step 1: Samples are collected from the sources.

Step 2: In conical flask 25ml of Agar solution has been prepared for the bacterial culture formation.

Step 3: The agar preparation is autoclaved and poured into the petri dish while it is still warm liquid so that bubble cannot be formed.

Step 4: Using a micropipette (range 10-100 μ l) 50 μ l of sample is taken and using a spreader it is been spread evenly on the surface of the media.

Step 5: The petri dish is then kept upside down and allowed to incubate to let the bacteria grow and form bacterial colony at right temperature and time. (37 degree Celsius and overnight).

Step 6: The next day three solutions of nutrient broth are prepared in conical flask for each sample. Each flask should be containing 10ml distilled water with nutrient broth powder. This is done for dilution purpose and measuring absorbance.

Step 7: With the help of an inoculation loop visible amount of bacterial colony has been taken in one of the three conical flask and rest two are kept as blank and dilution purpose.

Step 8: Conical flask having the bacterial culture is kept in the shaking incubator overnight. (Note: The speed of the shaking motion should be at a constant rate. Nor interruption or break can cause problem in the bacterial culture growth)

Step 9: After that the next day the conical flask with bacterial culture is removed from the incubator and placed inside the laminar flow cabinet. 2-3ml of solution from that flask has been taken in cuvette.

Step 10: Another 2-3ml of nutrient broth solution is taken in another cuvette as blank which has been prepared earlier. First the UV spectroscopy should be standardized using blanks in both the cuvette and then one of the blank has to be replaced by sample.

Step 11: Now those two cuvettes have been placed to measure the absorbance with the UV spectroscopy. (Note: The wavelength should be 600nm and cuvettes have to be rinsed properly and should be sterilized with ethanol after use)

Step 12: After getting the absorbance it is been used to measure the MIC (minimum inhibitory concentration) amount of bacterial culture in microliter that needed to be diluted in the nutrient broth solution which has been prepared earlier.

Step 13: Using the micropipette, desired amount of solution is taken from the conical flask of which absorbance has been measured and transferred into the conical flask containing 10ml of nutrient broth for 2nd time dilution.

Step 14: Then three petri dishes following proper concentration level in the container are prepared with Muller Hinton agar and set properly.

Step 15: A sterile cotton swab has been dipped in the 2nd diluted solution and spread on the surface of the petri dish evenly containing Muller Hinton media.

Step 16: Antibiotic disc are placed on top of the Muller Hinton media and one piece of autoclaved punched paper is also placed as blank for reference

Step 17: Now the petri dishes are placed inside the incubator for overnight at 37 degree Celsius to test antibiotic resistance.

Step 18: Next morning the petri dishes are removed from the incubator. Lysis indicates dead bacterial colony and diameter of the dead colony has been measured.

Step 19: Values of bacterial inhibition circle in mm diameter are measured using average of three values following standard deviation.

Precautions-

- Lab safety measures were followed strictly.
- Whole procedure has been done inside the laminar air flow cabinet.
- All the materials and instruments were autoclaved before use.
- All the weight of materials was taken carefully to maintain the concentration accurately.
- All the instruments were handled and cleaned carefully.
- Cuvette of the UV spectroscopy was rinsed and sterilized using ethanol before and after every use.
- Temperature and times were maintained carefully following standard protocol.
- Samples were used within 4 hours of collection.
- All the agar media were poured after shaking it properly before every time it poured while it was still warm liquid to avoid bubble formation and solidification.

- Antibiotic discs were kept inside the refrigerator as instructed.

2.6 Fecal Coliform Test

Before Starting-

- The temperature of the incubator was set at $44\pm 0.5^{\circ}\text{C}$.
- The temperature of the incubator was first stabilized and then samples added.
- Hands need to be sanitized with soap and water.
- The working station has to be sterile and cleaned with germicides, bacterial spray, and weak bleach prior before starting the procedure.
- All the materials are made sterile which come in contact with the sample.
- The vacuum has been removed as soon as the funnel is empty during filtration so that the filter does not become dry.

Materials and Ingredients

- mFC agar
- Bacto Agar
- Distilled water
- Rosalic Acid
- Petri dish
- Membrane filter, 0.45 micron
- Incubator
- Pipets

Preparation of FC plate

Step 1: At first 37 gm mFC powder was taken in a conical flask.

Step 2: Then 15.6 gm of Bacto Agar was added to it.

Step 3: 1000 ml distilled water was added to the powder and agar.

Step 4: 10 ml of Rosalic Acid solution was added with the mixture.

Step 5: The mixture was mixed well.

Step 6: Boiling of the mixture in a heater and was cooled at room temperature.

FC count

Step 1: At first 100 ml of the sample was filtered with 0.45 nm filter paper.

Step 2: The filter paper was put in the FC plate.

Step 3: The plate was placed in the incubator at 44.5°C for 24 hours.

Step 4: Finally the blue colonies were counted which indicated FC colonies.

2.7 Total Coliform Test

Preparation of TC Plate

Step1: At first 37 gram of MTC powder is taken into a conical flask.

Step 2: Then 1000 ml of distilled water is added so that the MTC powder is dissolved.

Step 3: The mixture is stirred gently to dissolve the solute.

Step 4: The mixture is then boiled for a couple of minute.

Step 5: After boiling the mixture for a while it is kept aside to cool down slightly.

Step 6: The light warm mixture is then placed in an absorption pad.

TC Count

Step 1: 100ml of sample is filtered by 0.45µm filter paper.

Step 2: The filter paper is then placed on the absorption pad which has the MTC mixture.

Step 3: The absorption pad is placed on a plate and placed in the incubator at 37 degree Celsius for 24 hours.

Step 4: The next day counting of the dark blue/purple/black colony are done.

Chapter 3

Results

Table 1: Result of Fecal Coliform Counts from the Central Lab, Mohakhali Dhaka.

Sample Source	Water Quality Parameter	Bangladesh Standard	Concentration Present	Unit	Analysis Method
Filter	Coliform (Faecal)	0	0	N/100 ml	MFM
Filter	Coliform (Faecal)	0	8	N/100 ml	MFM
Filter	Coliform (Faecal)	0	36	N/100 ml	MFM

From the data in Table 1, two of the samples are found to be contaminated by FC as revealed by the fecal coliform plate which indicates the presence of pathogenic bacteria. One sample out of the three has been found to be meeting the standard set by World Health Organization (WHO) which is zero coliform per 100ml of water sample. Other two samples are found to be moderately risky as the level is 10-100 per 100ml. As Bangladesh standard for FC is also zero as WHO standard so one sample could meet the standard. Previously in summer, a similar FC test of these three samples had been conducted and they showed more than 100 coliform per 100ml which was a high risk for consumption yet this time (in rainy season) they showed moderate risk level.

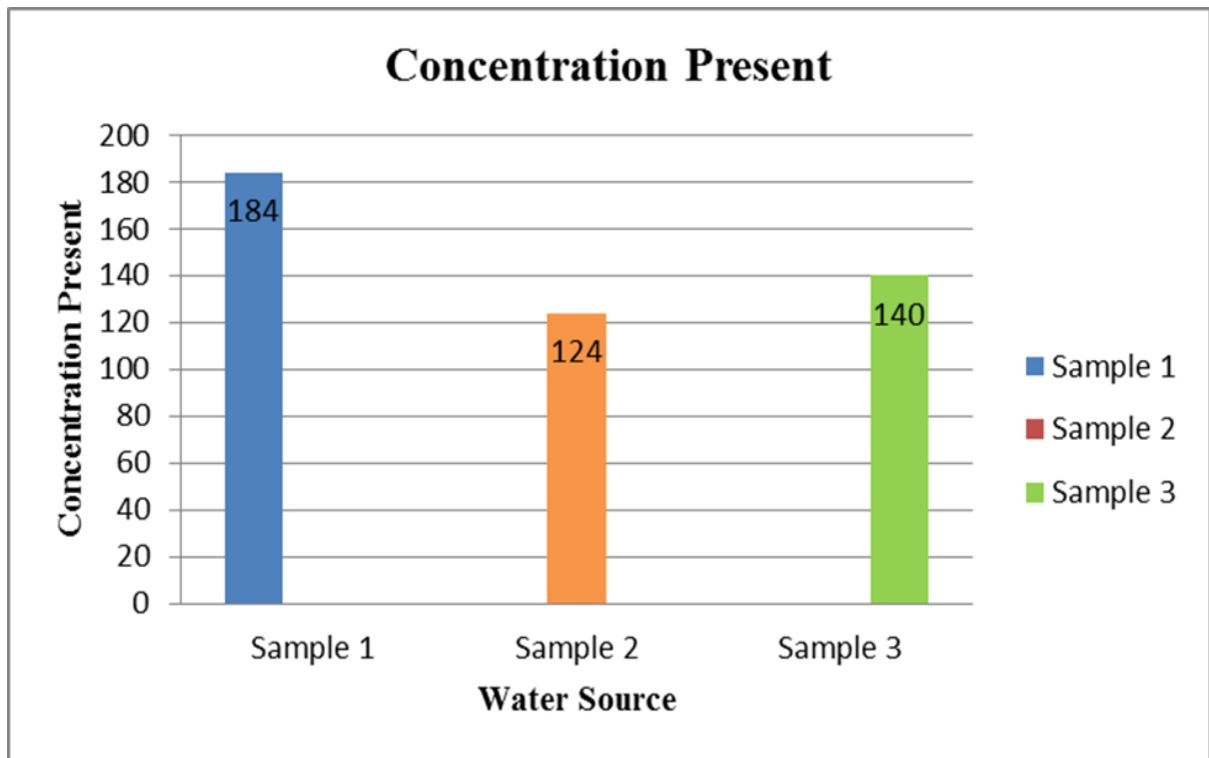


Figure 3: Bar chart showing FC count of previous survey conducted

Figure 3 represents a survey study previously conducted by a student using water sample of filter water from some selected source where these three values were found as high risk for consumption. Those samples were collected in the summer season of the following year so to test the FC count of those sources in different of time and season further testing were conducted as followed by the Central Lab. This could lead to a final verdict about the purity of the samples as different time were chosen so that it can be considered whether it is the time or season that lead to the results to be contaminated or the sources are contaminated from the very beginning and continued to be risky for direct consumption for human.

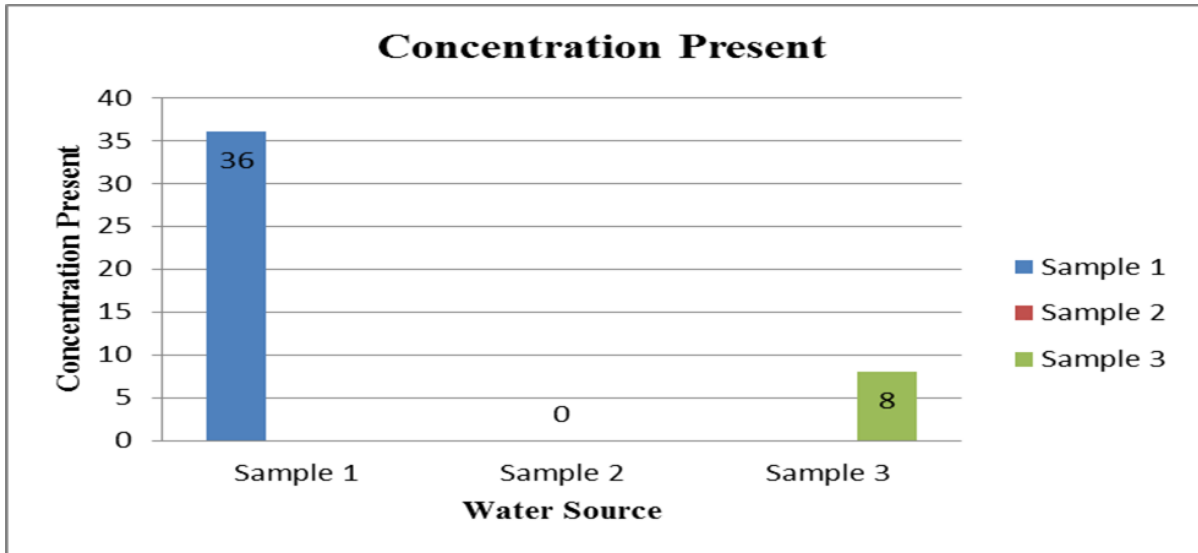


Figure 4: Bar chart showing FC count of the three high risk samples.

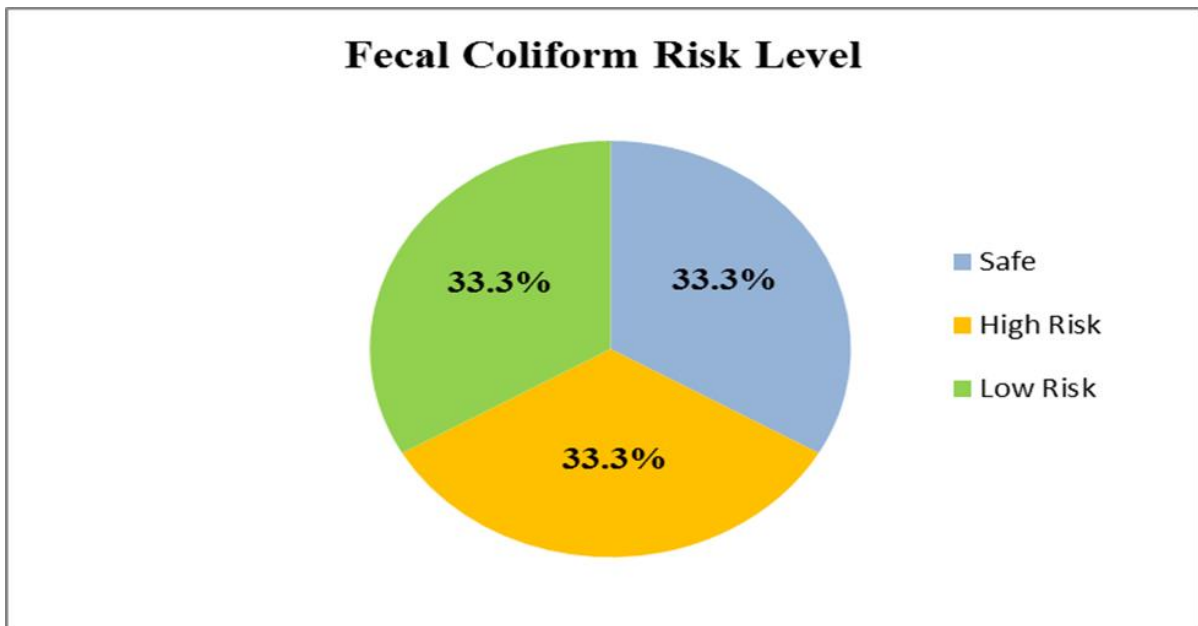


Figure 5: Risk Percentage of Fecal Coliforms from Central Lab results.

According to Figure 5, FC risk level has been categorized in percentage based on the result obtained from the Central Lab, Mohakhali. As seen the figure, only three samples are performed the FC test among them only one is safe and meets the standard of Bangladesh and WHO whereas other two are moderate to risky level. As only three samples are selected for the FC test, the percentage of risk and safe level are considered equal.

Table 2: Result of Total Coliform Counts from the Central Lab, Mohakhali Dhaka.

Sample Source	Water Quality Parameter	Bangladesh Standard	Concentration Present	Unit	Analysis Method
Filter	Coliform (total)	0	400	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	550	N/100 ml	MFM
Filter	Coliform (total)	0	380	N/100 ml	MFM
Filter	Coliform (total)	0	610	N/100 ml	MFM
Filter	Coliform (total)	0	26	N/100 ml	MFM
Filter	Coliform (total)	0	120	N/100 ml	MFM
Filter	Coliform (total)	0	132	N/100 ml	MFM
Filter	Coliform (total)	0	0	N/100 ml	MFM
Filter	Coliform (total)	0	48	N/100 ml	MFM

From data collection of Table 2, ten samples of filtered drinking water are tested TC count conducted by the Central Lab, Mohakhali. Among the samples only two of them meet the standard of Bangladesh and WHO standard for TC count which is supposed to be zero color per 100ml of sample water. Also two of the samples have TC level of 10-100 per 100ml which is considered as moderately risky according to WHO classification. Rest six samples were found to be more than 100 up to 610 coliform which is a very high level of TC in filtered drinking water sample. Those are categorized in high risk level of TC following both Bangladesh and WHO standard.

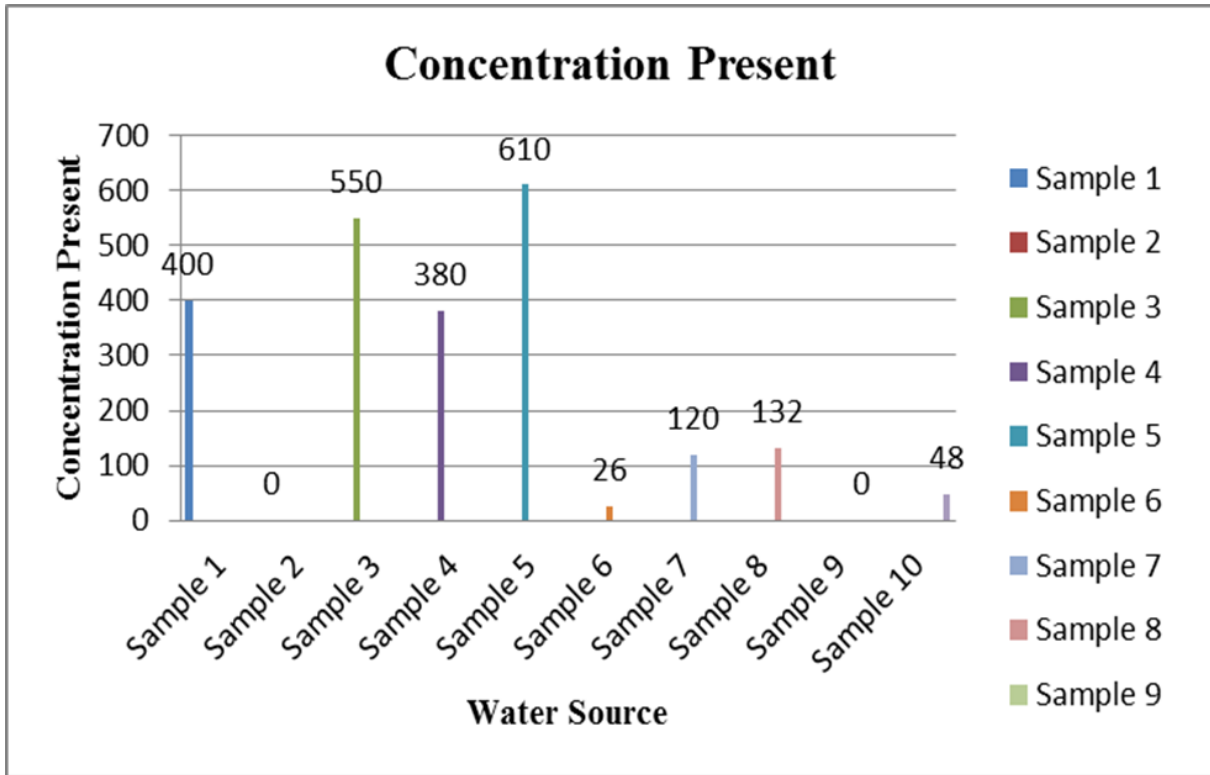


Figure 6 shows the results conducted by the Central Lab, Mohakhali of ten filtered drinking water samples collected from different sources around the Mohakhali area.

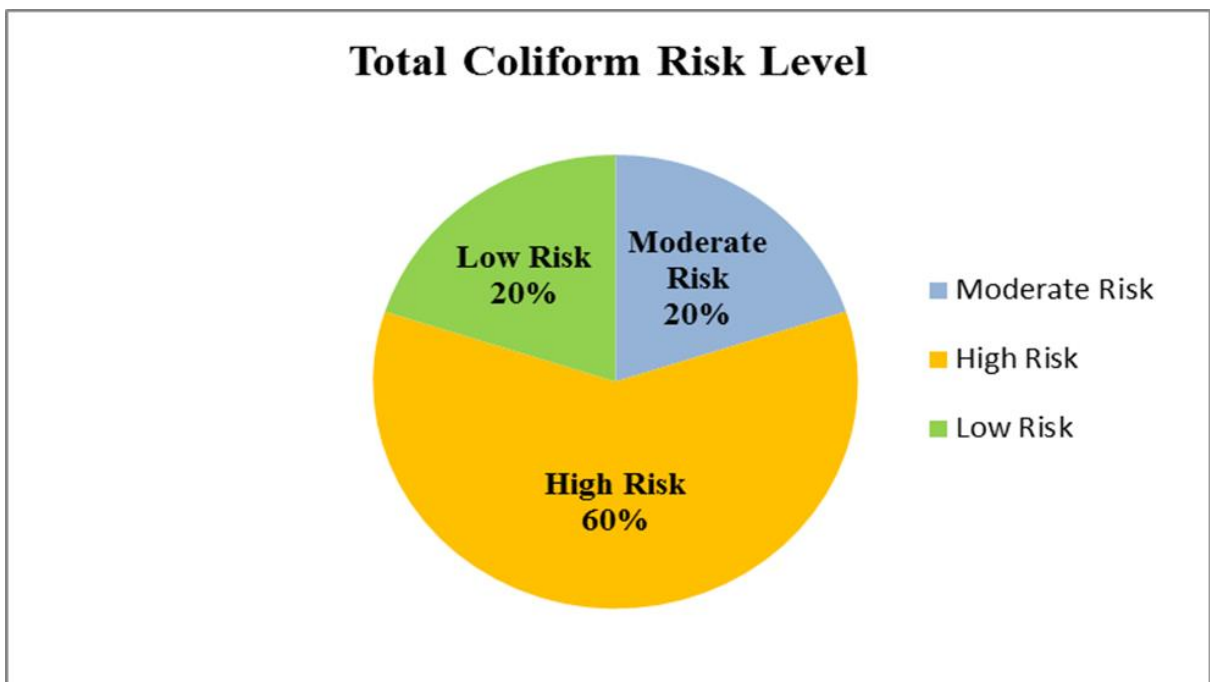


Figure 7: Risk Percentage of Total Coliform from Central Lab results.

According to figure 4, total coliform count has been categorized in percentage based on the results conducted by the Central Lab, Mohakhali. About 20% of the samples are found to meet the standard and 20% could be considered as moderately risky. Rest 60% samples are considered as high risk level of total coliform contamination following both Bangladesh and WHO standard.

Chapter 4

Discussion

This survey was confusing to identify the microbial contamination of the drinking water samples from the restaurants and tea-stalls around Brac University and Mohakhali area. The drinking water samples collected from the tea stalls have the same supplier and the water samples of the restaurants have mostly direct line of filtered water from the Water Supply and Sewerage Authority (WASA) which is the main body administering Water supply, Drainage and Sanitation system in Bangladesh. The three samples that are tested for fecal coliform are those stable restaurants around Mohakhali of which drinking water was been tested previously and found high level of fecal coliform in the water by one of the students of Brac University but the values of fecal coliform count are found very low this time. This can be the reason of seasonal change in sample collection as previously the samples were collected in summer and present samples are collected in rainy season. Though the fecal coliform count in rainy season should be more than other season as bacterial growth are higher in this period of the year yet results are very much acceptable in this case. Mostly they met standard of both Bangladesh and WHO which is zero coliform per 100ml of sample. If the location of the samples being collected are considered it is been noticed that not all class of people are found to be exposed to the same area of the region. So the people consuming water from areas around Mohakhali should be more careful about from where they are taking those waters at least for direct consumption.

In case of total coliform count, out of ten samples only two of them met the standard for both Bangladesh and WHO which is zero for every 100ml of water. Other eight of them have mostly shocking results. Six of them have more than 100 coliform per 100ml of sample water which total unacceptable. These might be the reason behind contaminated pipelines or leakage in these pipelines through which airborne microorganisms enter into the water and contaminated it. More than half of the samples are found to be at high risk of consumption. An effective solution for this might be maintaining of the pipelines, regular inspection of all the transporter of water and most importantly raising public awareness among the community. Also companies that supply the dispensing water containers should be identified and tested whether it is the container or the main water that has been supplied are contaminated and also the dispenser should be tested to have a clear idea from where the contamination is arising. Any carelessness in this regard can be considered as an influencer for the contamination of the water sources. The samples collected from wireless gate were safer than samples collected from area around Brac University premises. So almost all the students of Brac University are at high risk level. Six of the samples collected were the most visited stable restaurants and small hotels alongside the campus buildings where almost every student has their meal of different times. So the students of Brac University are at a vulnerable place as they are exposed to consume water for drinking is highly contaminated with bacterial coliform.

Throughout the survey one important thing was noticed that all the container water that were supplied to the tea-stalls or small hotels have different results both in fecal coliform and total coliform count which indicates that probably the supplied water is not totally contaminated from the beginning, it can be contaminated while dispensing or transporting to the shops. Unhygienic environment around the shops can also be an important aspect of variety in the results. Another important issue which was raised that the water inside those containers are

actually collected regularly from the supplier or they kept the container and filled it with tap water or any other sources to get the containers filled as it costs an amount of money to get the supplier water. So all the aspects should be considered equally for better understanding of the safety of the drinking water that are consumed by a large number of people around Mohakhali area including students of Brac University.

Chapter 5

Conclusion

Water is the core element to function the human body as well as other animals. The safety of drinking water is the least one can expect as it is one of the basic human rights. Because of the unhygienic environment and poor maintenance of the sewage and industrial waste management system in Bangladesh, there is a huge lack of fresh or improved water sources. As a developing country like Bangladesh where minimum health care for the whole nation is not strong enough there the increasing waterborne diseases are a serious threat. The main water system of Bangladesh WASA should be taken under consideration as the Central Lab; Mohakhali has showed differences in both cases of FC and TC count. Also the pipelines that transport water from WASA to different restaurants need to be tested for clarification of the water contamination that is considered filtered.

Chapter 6

Future Work

The level of total coliform count in most of the restaurants and tea-stalls are found very high. Those samples need to be tested for the specific microorganisms. Since the fecal coliform result fluctuated in different seasons of the year, further study on FC count can be done. Also other parameters that can define the actual reason behind the coliform contamination should be taken on account.

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