A Survey on Fecal Coliform Count and Ammonia Concentration Using Tap and Filter 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 (Hons)

Department 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 Ammonia Concentration using tap and filter water samples collected from tea-stalls and Roadside Restaurants of Mohakhali, Dhaka''submitted by Afrida Andaleeb Shahid of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 22.08.2019.

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Ethics Statement

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

Abstract

Twenty water samples from road-side restaurants and tea-stalls were collected following the protocol of collection of water samples for the analysis Fecal Coliform Count and Ammonia Concentration by Membrane Filtration Method (MFM) and Ultra Violet Spectrophotometry (UVS). The tests were conducted by The Central Lab of Department of Public Health Engineering, Mohakhali Dhaka. Eleven samples (55%) are found to be moderately risky as per WHO and Bangladesh standard (N=0/100 ml), six samples (30%) are less risky and three samples (30%) are highly risky. The level of Ammonia concentrations of all the samples were within the acceptable limit of Bangladesh standard (0.5 mg/L). Also, antibiotic resistance of the samples were performed using Amoxicillin and Azithromycin which are Penicillin antibiotic and Macrolide antibiotic, respectively.

Keywords: Fecal coliform; Ammonia concentration; MFM; UVS; Amoxicillin; Azithromycin.

Dedication

Dedicated to my grandparents.

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

ARB	Antibiotic Resistant Bacteria
ARG	Antibiotic Resistant Gene
FC	Fecal Coliform
FIB	Fecal Indicator Bacteria
JMP	Joint Monitoring Program
LIMC	Low and Middle Income Countries

Chapter 1

Introduction

The role of water is indispensable and it is the elixir of our existence. Water plays a vital role in every sector of life. Not only in quotidian uses, it is also necessary in many factories such as, chemical industry, beverage industry and also cosmetic industry (Mishra et al., 2018). Looking back into the history proves that fresh water is essential as the civilization flourished by the banks of rivers, the biggest source of fresh water. However, due to the pollution of water its indispensability seems to be handicapped leading to unusable. Huge amount of antibiotic resistant bacteria from hospitals, clinics, animal husbandry and public defecation pollute water due to improper drainage system. These bacteria can end up by affecting humans and livestock (Ahmed, Clegg, Williams, Baptiste, & Bennett, 2010); (Crimmins & Beltrán-Sánchez, 2011);(Carlet et al., 2012). Waterborne diseases are included in leading reasons for the death of the children who are under the age group 5 (*Global Water Supply and Sanitation Assessment 2000 Report*, 2000); (Howard & Bartram, 2003). A number of bacteria was chosen for the indicators of water potability from Enterobacteriacea (Fewtrell, Bartram, Ashbolt, Grabow, & Snozzi, 2001)(Behera & Mishra, n.d.-b); (Behera & Mishra, n.d.-a) family. These bacteria reproduce in the gut of mammals having warm blood, indicate the existence of other pathogens and can be detected in feces. These bacteria contain drug resistant genes and also have the ability to transfer them to other nearby pathogens in a very short time. Thus, they pose as health hazards. Therefore, monitoring of fecal coliforms level of water has become a necessity in order to reduce health hazards (Mishra et al., 2018).

1.1 Diseases Related to Impure Household Water

Diseases caused by polluted water which are used for drinking are responsible for creating major pressure on public health. Infectious diseases for example- diarrhea, cholera, dysentery,

other diseases similar to diarrhoea and enteric fever can occur by drinking water which has feces and pathogens (White, Bradley, & White, 2002); (*Guidelines for Drinking-water Quality FOURTH EDITION WHO Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4 th ed*, 2011). However, these waterborne diseases may vary as per context and it found to be highest among low-income people. Diarrhea is found to be the leading reason behind child death among the low-income mass (Liu et al., 2012). From birth, the next two years are the most important time in a child's life in meeting the nutritional requirements. During this time, a child needs extra nutrition for faster growth. However, in South-Asian countries a different scenario can be observed. In Bangladesh 25% of the children below two years are found to be underweight (Balows & American Society for Microbiology., 1991). Preparation of complementary food for children apart from breast feeding opens a door for enteropathogens. Thus, diarrhea is highest among the children under this age group and thus a negative effect falls on their nutrition (Mostafa et al., 2018). One of the studies conducted in Nigeria shows that children under the age group two years suffer from diarrhea among all the age groups. A similar study which was conducted in Bangladesh shows that 40% of the complementary food for children contained *Eschericia coli* (Mostafa et al., 2018). However, reviews of epidemiological proof from different studies show that the quality of drinking water plays a huge role in transfer of fecal coliforms in humans. It is not easy to recognize the side-effects of one single element and linked fecal-oral pathways. These pathways are to the point of contexts. (Bain et al., 2014).

1.2 Contribution of WHO/UNICEF/JMP

Joint Monitoring Program for Water Supply and Sanitation (JMP) of WHO monitors the worldwide access to pure drinking water and UNICEF follows the part of population using improved source for drinking purpose. Improved source can be defined by the source which is protected from contamination due to the nature of construction, particularly fecal substances (Kostyla, Bain, Cronk, & Bartram, 2015). This does not stand for actual contamination and it as a substitute of safety. However, it is a shortcoming since WHO defines 'safe drinking water' as water "which does not represent any significant risk to health over a lifetime of consumption" (*Guidelines for Drinking-water Quality FOURTH EDITION WHO Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4 th ed*, 2011). Furthermore, fecal contamination is regarded as the major warning for public health (*Report JMP Technical Task Force Meeting on Monitoring Drinking-water Quality*, n.d.).

It has been found from a systematic review that at least one-fourth of water samples from the improved sources of low income areas contain fecal contamination and 38% of water quality tests of the total report the same (Bain et al., 2014). The estimates show that 1.8 billion people collect drinking water from polluted sources (Bain et al., 2014); (Wolf et al., 2018); (Onda, Lobuglio, & Bartram, 2013).The JMP has considered the monitoring of fecal contamination as the next step in accelerating worldwide monitoring of pure drinking water (Aliev, Shodmonov, Babakhanova, & Schmoll, 2010). The monitoring of fecal pollution in drinking water is done through fecal indicating bacteria (FIB). At present, E.coli is recognized as the best FIB by WHO and JMP for monitoring fecal coliforms (*Guidelines for Drinking-water Quality FOURTH EDITION WHO Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4 th ed*, 2011). Also, according to WHO, TTC or thermo tolerant coliforms can be regarded as alternatives (*Guidelines for Drinking-water Quality FOURTH EDITION WHO*

Library Cataloguing-in-Publication Data Guidelines for drinking-water quality-4 th ed, 2011); (*Report JMP Technical Task Force Meeting on Monitoring Drinking-water Quality*, n.d.)

1.3 Drinking Water Standards and Improved Water Sources

As per the WHO guideline, in any 100 ml water sample the count of E.coli is "none detectable". Quantification has become simpler by using E.coli or thermo tolerant coliform (alternatively) and latest enzymatic procedures which are comparatively less costly, more robust and simpler. It is approved by WHO GDWQ. The WHO GDWQ also recommends that E.coli or thermo tolerant coliforms can be used alternatively in quantifying fecal contamination in drinking water (Bain et al., 2014). Maximum standards of E.coli in OECD member states and LIMCs follow the guidelines of WHO for E.coli. Furthermore, WHO GDWQ suggests to use risk classifications for highlighting interventions because higher level indicators indicate higher level of fecal pollution.

The most common risk classification which is used based on the count of the indicator organisms in 100 ml and it includes, 1, "very low risk"; 1-10, "low risk"; 10-100, "medium risk"; 100, "high risk" or "very high risk" (Clasen & Bastable, 2003), (WHO, 1998)Yet, FIB are not perfect as their level cannot help to determine the actual risk (*The Coliform Index and Waterborne Disease*, n.d.); since the quality differs with time and area. Also, due to infrequent sampling, the actual exposure of contamination might not be reflected.

One of the most useful ways of identifying hazards and preventing risk management is sanitary inspection. The water area and its surrounding needs to be inspected in this case (Lloyd & Bartram, 1991), (Clasen & Bastable, 2003)(WHO, 2009). Improved indicator works in very simplified way for sanitary inspections. Sanitary inspection has been a widely used tool for assessing the quality of drinking water like FIB.(Bain et al., 2014). Forms which have been standardized are proposed for the assessment of sanitary risk along with derivation of a

summary measure which will be the sanitary score (Bain et al., 2014).The forms usually have questions regarding integrity of protective objects for example, well covers or fencing and the probable source of hazards like latrines. Sanitary risk factors might also vary like water quality depending on time and area. This approach can be merged with microbiological analysis to obtain a risk cross-tabulation. (Lloyd & Bartram, 1991) or as a section of highly detailed water safety plan (Davidson et al., 2005).

1.4 Improved Indicators of Fecal Coliforms

The WHO and UNICEF merged together in January 2012 in order to improve targets and indicators. The aim was to perform monitoring of drinking water, sanitation and hygiene by the year 2015 in a better way. The group working with water suggested the continuation of using improved water source classification. It would be used as one of the parts of the revised group of indicators (Analog Devices, 2013). In developing countries this level of sample collection and testing is not possible due to limited resources. Instead, FIB monitoring is done often through infrequent sampling, using few samples a year (Onda et al., 2013). Due to untypical sampling timing, the accuracy of fecal contamination can be impaired in areas since FIB can be present in infrequent contamination. Adding up, microbial contamination can vary with time. Also, FIB survives no longer than four to twelve weeks (Edberg, Rice, Karlin, & Allen, 2000).

1.5 Seasonal Variation Fluctuates the FC Count

Season can be highlighted as the major concern in case of sampling. It can be divided astronomically (eg: summer, winter, autumn) or as per climate (eg: dry, wet). Within the context of this survey, season refers to dry and wet seasons. According to WHO, the quality of water is worse during wet season thus the variation of seasons is an important issue (Onda et al., 2013). However, drinking water surveys are usually conducted in dry season due to ease of

movement. Less fecal contamination due to inaccuracy and misleading is possible to obtain if seasonal trends are considered. The seasonal trends can help to polish the guidelines of sampling and thus help in enhancing the available data. A trend having known effects can help to interpret new trends. Previous studies show that water quality parameters of surface water followed seasonal pattern (Ouyang, Nkedi-Kizza, Wu, Shinde, & Huang, 2006). Again, in studies conducted in developing countries and unimproved water sources show that they have also used seasonal patterns (R. C. Wright, 1986). However, no review has been conducted on general or basic seasonal pattern on fecal contamination from the source of improved drinking water in developing countries. (Kostyla et al., 2015)

1.6 Drinking Water Sources

More than 90% of the total world population use improved sources for collecting drinking water among which half of the population collect from pipe on premises (Bain et al., 2014). Improved water sources are assumed to protect the water from contamination to some extent. However, a portion of the water quality data from low to middle income countries, which is 38%, have reported to contain fecal coliforms (Bain et al. 2014). Furthermore, there is a possibility of the presence of fecal coliforms in more than 10% of sources labelled as improved sources, more than 100 colony forming units/100 ml (CFU/100 ml) (Bain et al., 2014). There is risk of contamination in storage area or through transportation even if the quality of water matches with WHO Guidelines (not detectable in 100 ml) (Bhunia, Ramakrishnan, Hutin, & Gupte, n.d.); (Alarcon Falconi et al., 2017); (J. Wright, Gundry, & Conroy, 2004). More than three hundred million people around the world collect tap water intermittently. The time lies between a few hours per day to a few hours per week. The average time of water supply is 7 hours per day in countries of South Asia (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). About 19% of urban slum dwellers have the access to household tap water in

India in comparison to 50% of total urban households. More than 20% of the slum dwellers collect water from the taps away from their premises at a distance of 100 meters or more (Alarcon Falconi et al., 2017). The sources through which municipal of India supplies water are found to be polluted and the level of contamination increases upon household storage (Alarcon Falconi et al., 2017); (Firth et al., 2010); (Trevett, Carter, & Tyrrel, n.d.). Thus, the chances of occurance of waterborne diseases increase (Prüss-Ustün et al., 2014); (Sarkar, Sivarathinaswamy, et al., 2013); (Sarkar, Ajjampur, et al., 2013).

Multiple studies have displayed the difference in the level of water contamination between the source of water and the households along with environmental parameters and behavioral factors at household-stage (Eshcol, Mahapatra, & Keshapagu, 2009), (Levy, Nelson, Hubbard, & Eisenberg, 2008), (Mintz E, Reiff F, & Tauxe R, n.d.). The most highlighted associations of high risk factors of water quality degradation from source to household are crowding by people or room, latrine usage and water purification (Kattula et al., 2015). The degradation of water quality depend on paired or separate sample collection (Shields, Bain, Cronk, Wright, & Bartram, 2015). Paired collection of samples provide detailed information including the source and probable sources through which the water might get contaminated in stored condition (Levy et al., 2008)In spite of a well-designed method of sample collection, the results might vary due to irregular supply of water in field condition. Studies in which source and household water are collected separately, gives flexibility at the time of water collection (Abdellah, Abdel-Magid, & Yahia, 2012); (Aliev et al., 2010). However, such type of study-design needs matching of sources with households (Shields et al., 2015) and generally contains less information on exact source of water and length of storage of household water.

1.7 Antibiotic Resistant Bacteria (ARB) in Water

ARB along with their genes have taken the place of flourishing contaminants, having a worldwide distribution (Pruden, Pei, Storteboom, & Carlson, 2006); (Diehl & LaPara, 2010); (Dodd, 2012); (Chen et al., 2015). They have natural characteristics which is helping in its dispersion in the environment (Allen et al., 2010); (Gaze et al., 2011); (Wellington et al., 2013).Due to the increasing demand of drinking water, contaminated water resources might be considered as drinking water source or contaminated source. Numerous water treatment methods are used for drinking water sources for the removal of contaminants. For example- sedimentation, disinfection, filtration and flocculation. Among all these, chemical disinfection is noteworthy (Khan, Beattie, & Knapp, 2016). It helps to reduce microorganisms from water treatment plants. However, chemical disinfection does not work effectively under multiple factors which reduces its effect (Scully, Hartman, Rule, & Leblanc, 1996); (Jaglic et al., 2012);(Cherchi & Gu, 2011). Chemical disinfection also reduces its effect against organic nitrogens (Scully et al., 1996) and extracellular polymeric matrix (Cherchi & Gu, 2011). It has been found that resistance spreads horizontally by cross resistance or co-resistance. Antibiotic resistant microorganisms have greater frequency of tolerance to chlorine (Templeton, Oddy, Rogers, & Leung, 2009). E.coli is found to grow even when chlorine is present. Genetic factors such as class 1 and 2 intergrons transfer numerous resistant genes. These could be reason behind such traits (Hsu et al., 2014); (Su et al., 2012). It has been found from waste water study reports that, the number of total bacteria is less, however, the number of resistant bacteria is more in waste water (Galvin et al., 2010); (Al-Jassim, Ansari, Harb, & Hong, 2015). A similar report might be found in drinking water system as well. Reports on drinking water treatment plants (DWTP) and drinking water distribution systems (DWDS) show the emergence of ARB. It has been found that relative abundance of sulphonamide resistant genes have accelerated from 3.5% to 33% in DWTP (Chao et al., 2013) and a wider range of antibiotic resistant

genes(ARG) have been found. The areas having higher pH, salinity oxidation, less nutrient and exposure to chlorine promote greater ARB (Ridgway & Olson, 1982). Sub-inhibitory concentrations can increase a stress which might promote genetic exchange. Also, local disruptions in the main zones of distribution are responsible for microbiological populations. It may include waterborne disease agents (Wang, Edwards, Falkinham, & Pruden, 2013).

According to previous reports, ARB and ARGs are present in water distribution systems and in the drinking water sources (Bergeron, Boopathy, Nathaniel, Corbin, & LaFleur, 2015); (Coleman et al., 2013); (Fernando et al., 2016); (Guo, Li, Yang, Yang, & Yin, 2014); (Jiang et al., 2013). A study in India reported that fecal coliform strains were present in drinking water water sources and were resistant to 37 antibiotics (Skariyachan et al., 2015). Furthermore, different ARGs are responsible for the resistance to tetracycline, penicillin, chloramphenicol and sulfonamide, which are found in drinking water sources (Lyimo, Buza, Subbiah, Smith, & Call, 2016); (Bergeron et al., 2015) and DWTPs (Bai et al., 2015); (Jiang et al., 2013); (Guo et al., 2014). Biological activated carbon (BAC) filtration and chloramine disinfection in DWTPs could accelerate the resistance of ARB against ampicillin, kanamycin, rifampicin, chloramphenicol, and streptomycin (Bai et al., 2015). BAC filtration increased the number of ARGs through detection. Then chloramine disinfection increased the relative abundance of ARGs in the water generated from the DWTPs (Xu et al., 2016). Also, filtration by sand increased the relative abundance of ARGs, however decelerated the absolute ARGs concentration (Xu et al., 2016). In spite of the studies, it is not easy to understand how ARGs can be removed from DWTPs. So, it is very important to understand the variation of ARGs in tap water.

1.8 Environmental Fate Due To Ammonia

Hydroxyl ions and ammonium cations are formed on the dissolution of ammonia in water. In the reaction, equilibrium constant KB is, 1.78 × 10-5 (Dehnicke, 1996). Temperature,

concentration of the salts and pH of water is responsible for the degree of ionization. The nitrogen cycle is depended on nitrate, then ammonia and then ammonium cation which is dominating in nature. Ammonium cation is not very mobile in soil and water. In this case, ammonia is more mobile than ammonium cation. Ammonium cation however, is involved in nitrogen fixation, nitrification and other biological processes.

1.9 Analytical Method of Ammonia Concentration

Through indophenol reaction, the concentration of both ammonia and ammonium cation can be determined in between 0.025-3 mg/L (World Health Organization. & International Program on Chemical Safety., 1986). Ammonia selective electrodes and titrimetry which is not very sensitive can also be used.

1.10 Environmental Level and Human Exposure

Air

The air of urban areas might contain ammonia upto 20 µg of ammonia per mAir. In farm areas where animals are reared in high number, the concentration of ammonia in air might be around 300 µg/m3.

Water

Ammonia concentration in underground natural water is normally below 0.2 mg/L. However, higher concentration can be found in strata rich humic materials water which can be upto 3 mg/L. The concentration level of surface water might be upto 12 mg/L. Ammonia might be traced in drinking water due to disinfection with chloramines. If the level of ammonia is higher than the expected value, then it indicates fecal pollution. Abnormal taste and odor indicates higher level of ammonia concentration. This odor and taste can be observed when the water is disinfected with ammonia which is higher than 0.2 mg/L (World Health Organization. & International Program on Chemical Safety., 1986). High concentration of ammonia in water

does not let manganese removal filters work properly. Thus, water tastes earthy and looks moldy. Ammonia in raw water might end up with drinking water containing nitrites which are formed by the catalytic action of the filters which are caused by ammonium-oxidizing bacteria.

1.11 Approximate exposure and contribution of drinking-water

The approximate daily intake of by food along with drinking-water is 18 mg. However, by inhalation process, it is not more than 1 mg, and through cigarette smoking (20 cigarettes/day) also less than 1 mg. In contrast, 4000 mg of ammonia are produced endogenously in the human intestine each day

1.12 Kinetics + Metabolism of Ammonia in Laboratory Mammals

Mammal's one of the most key metabolite is Ammonia which plays an important role in acid–base regulation along with the biosynthesis of purines, pyrimidines. As a metabolic in nerve excitation and muscular activity liver produces this acid also Ammonia works in the digestive tract through enzymatic breakdown of food particles along with the help of bacterial flora [Source: Hazardous Substances Data Bank: Ammonia. Bethesda, MD, National Library of Medicine, 1990].. Blood absorb urea formed in the liver, transfers to the kidney and eventually turns it into urine. The amount of ammonia that can be found in urine its two third originates from the tubular epithelium of the kidney. In this case, ammonia is a product of glutaminase reaction and maintains the acid-base equilibrium by taking Hydrogen ions.

1.13 Effects of Ammonia on Humans

Human health has a toxic effect from ammonia if its intake becomes higher than the capacity to detoxify. Ammonia that is consumed in its form of salt, anion effect has to be considered. The acidotic effects of the chloride is of greater importance than ammonium ion with ammonium chloride. A dose more than 100mg/kg of body weight per day (33.7 mg of ammonium ion per kg of body weight per day), metabolism gets influenced by ammonium

chloride by shifting the acid-base equilibrium which creates glucose tolerance disturbance and also tissue sensitivity reduces to insulin.

1.14 Uses of Ammonia

Ammonia is a very common component in fertilizer. It is also used in animal feed production, in the manufacturing of plastics, paper, rubber, explosives. Also, it can be used as a coolant, in the processing of metal and as the reactant of numerous nitrogenous compounds (Dehnicke, 1996). Ammonium salts and ammonia are used as food ingredients and cleansing agent respectively. Ammonium chloride is a common diuretic [Source: Hazardous Substances Data Bank: Ammonium chloride. Bethesda, MD, National Library of Medicine, 1990].

The mixture of ammonia and water is a very useful fluid in the absorption refrigeration machines along with the absorption of heat pumps. Here, ammonia works as the refrigerant and water works as the absorbent. The mixture is reliable and safe to use as it does not harm the ozone layer. Also, it does not accelerate the greenhouse effect (Shamsetdinov et al., 2013).

Great effort was given in improving the working efficiency of thermodynamic cycles. Water and ammonia mixture was the first patent of the absorption refrigeration. Research is running on the working fluids and the elements of the process for example, condenser, generator etc (Sun, Fu, & Zhang, 2012). It is necessary to monitor different physical properties of the working fluid (eg- temperature, pressure, composition) for optimum usage, control and the whole process. Conventional RTD, thermocouples, transmitters helps to monitor the temperature and pressure easily. However, it is not easy to detect the composition of the mixture. The concentration of ammonia can be measured through external measurements or sampling but it is very time consuming. Also, the process is not possible to handle in real time. “In-situ” measurement can be an alternative at this point. Like, Coriolis flow and density meter. This meter is commonly used and gives accurate results. Also, it can calculate the fluid density.

In case of unknown temperature and pressure, the composition can be explored through previous data (Barba, Berdasco, Salavera, Larrechi, & Coronas, 2017). If multiple devices are needed for the determination of the ammonia concentration then the cost is higher. Therefore, it is a challenge to run absorption refrigeration economically and with accurate composition and concentration. Also, for this reason, this field is a research prone sector (Barba et al., 2017). A review on potential sensing methods which might be helpful for the measurement of the ammonia/water concentration in absorption refrigeration cycles. The reviewed measuring criteria includes the acoustic wave, refractive index, optical absorption and electrical conductivity.

At present, sensors are great means for tracing ammonia. However, the experiments conducted so far have been performed in high concentrations in the working fluid of absorption refrigeration which is water/ammonia mixture (Barba et al., 2017).

1.15 Objective

A large group of people in Bangladesh, living in main cities and towns have their meals at roadside restaurants frequently. Nowadays, mirobiological safety in water for drinking purpose is a burning issue and people are gradually getting to know about waterborne diseases through awareness. Consumers are now forced to look for safer option due to multiple incidents of municipal water supply contamination. These contaminations take place from different external sources; also low maintainace of pipe-line results in leakage of pipes through which water passes. In such case, water bottles supplied by various companies become an option but they are costlier than the municipal supplied water. However, a low cost alternative is the water provided by companies for drinking purpose in big sealed containers. These containers are attached to dispensing machine directly. Water is provided to the consumers in small glasses after dispensation.

Since drinking water from dispensing machines is very common and popular in recent times, it has become a necessity to check the microbiological quality of water collected from dispensing machines. There is only one data of microbiological quality from dispensers in Bangladesh by Moniruzzaman et al. in which the count of TC, FC and HPC has been obtained. "BRACU Express", a monthly newspaper published by the students of BRAC University has published in November 2018 about budget friendly restaurants for university students around BRAC University area. Every student of BRAC University has visited these budget friendly hotels or restaurants at some point. For this reason it has become a necessity to perform microbiological tests of the samples collected from the restaurants near to the university premise. Our goal is to count the fecal coliforms in 8 drinking water samples and 12 piped water samples from different tea-stalls and restaurants of Mohakhali, Dhaka.

Chapter 2

Methodology

2.1 Site Location and Area

Mohakhali is a renowned commercial area where numerous Government banks, private banks, private firms and educational institutions are present. More than three hundred hotels and restaurants, including food carts are available in this area.

2.2 Sampling Site and Sources

Twenty water samples from tewnty different restaurants and tea-stalls of Mohakhali were collected to count Fecal Coliforms. The most busy restaurants and tea-stalls were given priority during selection of sources. Samples were collected from 13.06.2019- 30.06.2019. From two types of sorces, water was collected- tap water which is supplied by Water and Sewerage Authority, WASA and filters which are provided at tea-stalls for drinking purpose.

Figure 1 represents the location of restaurants and tea-stalls around Mohakhali area. The students of Brac University are exposed to these tea-stalls and restaurants and every student visit restaurants or tea-stalls among the located ones. The coordinates of the location are used to get the map using ArcGIS (Version 10.5). Architecture Geographic Information System which is known as ArcGIS, is a common software used by urban planners and geographers. It serves the purpose of working with maps and geographic information.

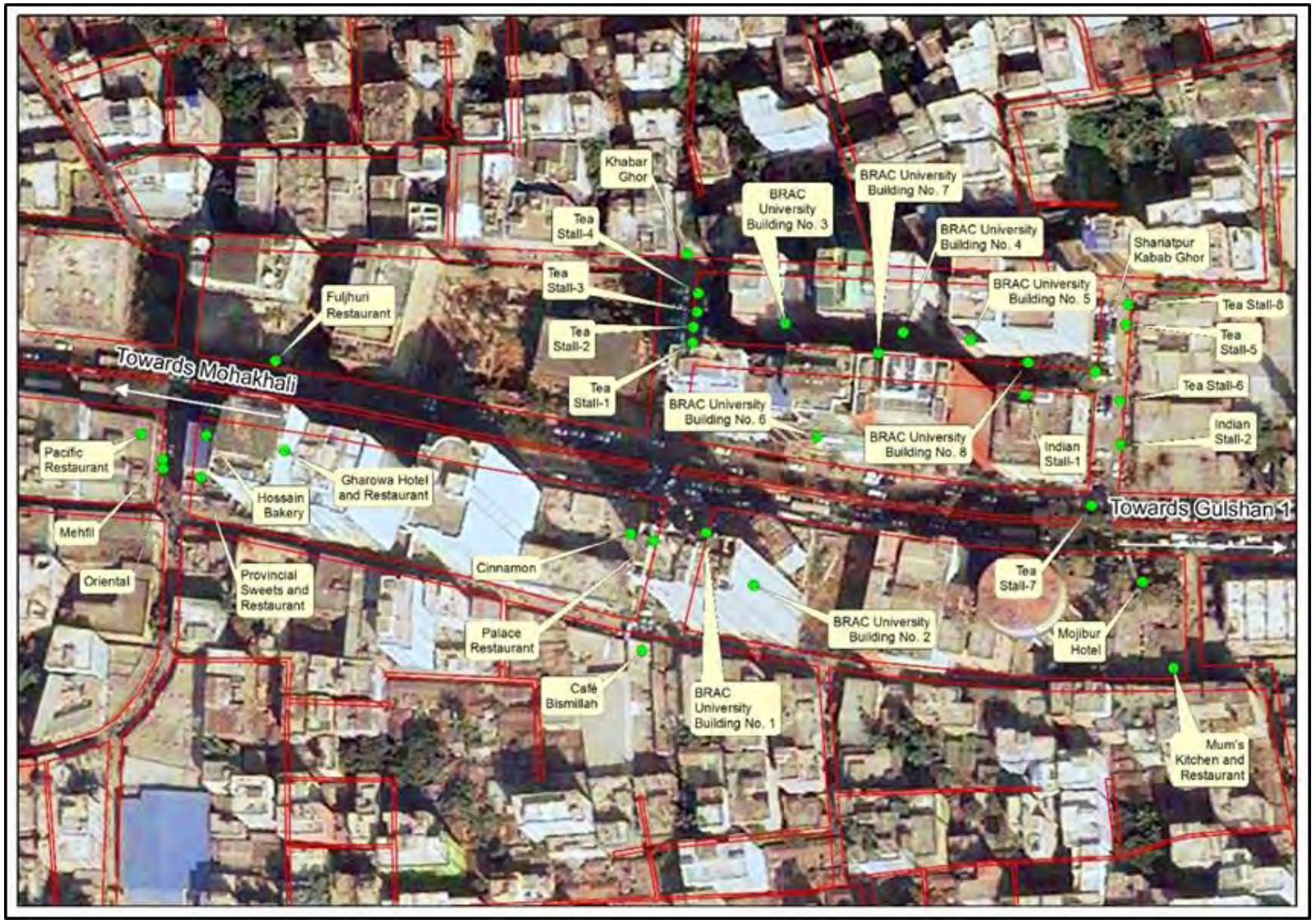

Figure 1: Map representing restaurants and tea-stalls around Mohakhali using Arc GIS.

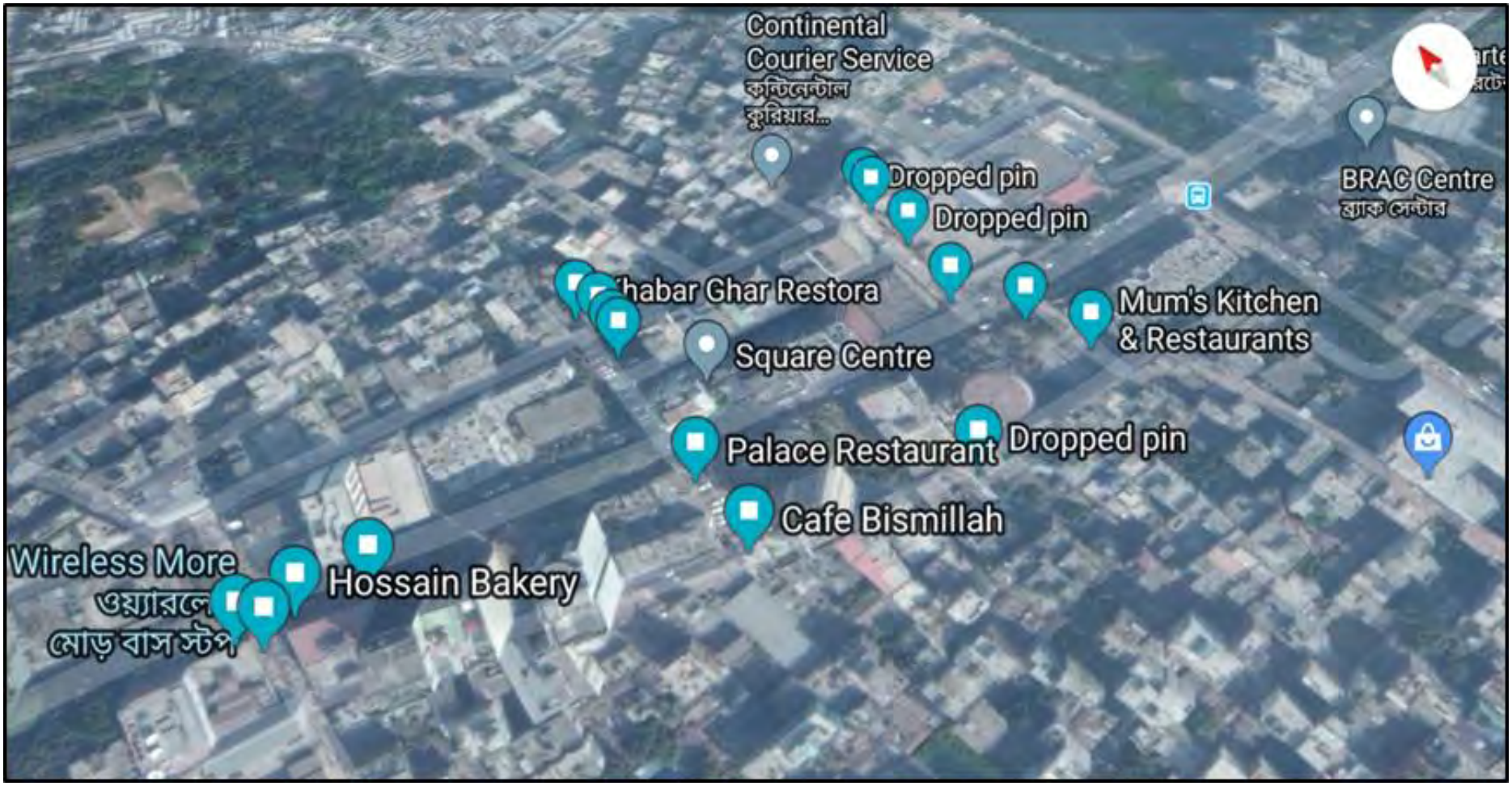

Figure 2: Map of Sampling Site from Google Earth

In Figure 2, only the restaurants and tea-stalls are plotted in the map derived from google earth. Using the coordinates of the sampling location, the restaurants are plotted in it. The pointed restaurants of figure 2 are also included in Figure 1.

2.3 Collection of Water

Water from different sources was collected following WHO-recommended procedures (WHO 1984). For example, the water tap was first wiped, using a clean cloth. Then the tap was turned on at maximum flow rate and allowed to flow for 2 min. The interior of the tap was sterilized using alcohol. Then 250-ml water samples were aseptically collected in sterile polyethylene terephthalate plastic bottles. The filters were treated similarly. All samples were transported directly to the Central Lab of Department of Public Health Engineering in an insulated box filled with cool packs.

2.4 Antibiotic Resistant Test

Azithromycin fights bacteria. It is prescribed to treat various types of infections which are caused by bacteria. For example, Skin infections, ear infections, eye infections and sexually transmitted diseases or STDs. It should be avoided if one suffers from jaundice or liver issues upon the intake of azithromycin.

Amoxicillin and clavulanic acid are used in combination in order to treat treat certain infections caused by bacteria, including infections of the ears, lungs, sinus, skin, and urinary tract. Amoxicillin is under the class of medications called penicillin-like antibiotics. It functions through stopping the bacterial growth. Clavulanic acid is under the class of medications called beta-lactamase inhibitors. It works by preventing bacteria from destroying amoxicillin.

Antibiotics do not work for colds, flu, or other viral infections. Using antibiotics when they are not needed increases your risk of getting an infection later that resists antibiotic treatment.

The samples were collected and refrigerated in -30 degree Celsius. No bacterial colony is supposed to be present if stored under this temperature. However, bacterial culture was found using Nutrient Agar plates and after testing with azithromycin and amoxicillin, all the samples was found resistant to the antibiotics. The antibiotic resistant test was done in the Microbiology Laboratory of the Department of Pharmacy, BRAC University following the standard protocol.

Sample is collected from the source

25ml of Agar solution has been prepared to let the bacterial culture formation

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

Using micropipette (range 10-100µl) 50µl of sample is taken and with a spreader it is been spread evenly on the surface of the media

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)

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.

With the help of a 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

Conical flask having the bacterial culture is kept in the shaking incubator overnight

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

(Continued)

in the cuvette. 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.

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)

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.

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.

Then three petri dishes are prepared with Muller Hinton agar and set properly.

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.

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

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

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

Values of bacterial growth in mm diameter are measured using average of standard deviation.

Figure 3: *Protocol of antibiotic resistant test performed in Microbiology Lab of Department of Pharmacy, Brac University*

Precautions-

- Lab safety measures should be followed strictly.
- Whole procedure has to be done inside the laminar air flow cabinet.
- All the materials and instruments should be autoclaved before use.
- All the weight of materials should be taken carefully to maintain the concentration accurately.
- All the instruments should be handled and cleaned carefully.
- Cuvette of the UV spectroscopy should be rinsed and sterilized using ethanol before and after every use.
- Temperature and times should be maintained carefully.
- Samples should be used within 4 hours of collection.
- All the agar media should be poured while still warm liquid to avoid bubble formation and solidification.
- Antibiotic discs should be kept inside the refrigerator.

2.5 Fecal Coliform Test

Before Starting-

- The temperature of the incubator has to be 44±0.5°C.
- The temperature of the incubator has to get stable, then samples are to be added.
- Hands need to be cleaned with soap and water.

- The working station has to be cleaned with germicides, bacterial spray, and weak bleach prior before starting.
- All the materials has to be sterile which come in contact with the sample.
- The vacuum has to be 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

37 gm mFC powder was taken in a conical flask.

15.6 gm of Bacto Agar was added to it

1000 ml distilled water was added to the powder and agar

10 ml of Rosalic Acid solution was added with the mixture

The mixture was mixed well

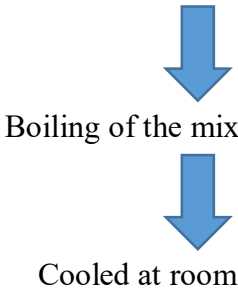

Figure 4: Preparation of FC plate

FC count

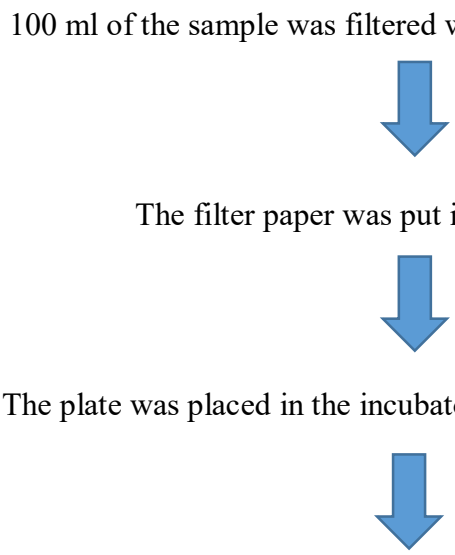

Figure 5: Protocol of FC count

Source: Central Lab, DPHE

2.6 Ammonia Test

Microbial protein catabolism of organic substances is responsible for the production of ammonia. Thus, high concentration of ammonia can indicate the decomposition of raw meat, milk, fish etc. Off-odors indicate higher level of ammonia since it has a pungent smell. Also, its concentration is high in urine and feces.

In this survey, Ultra Violet Spectrophotometer is used for the determination of the ammonia concentration. The test is done by the Central Lab,Department of Public Health Engineering, Mohakhali Dhaka. The samples were collected similar to the samples for the fecal coliform tests.

The motto behind checking ammonia concentration test is to find out whether waste materials are present in the samples.

Ingredients and materials

- Phenol-sodium nitroprusside solution
- Sodium hypochlorile
- Volumetric Flask
- UVS

Procedure

25 ml of the sample was taken in a 50 ml volumetric flask

10 ml of Phenol-sodium nitroprusside solution was added

De-ionized water was added up to the mark of 50 ml

1 hour waiting for the color development

The absorbance was measured at 640 nm

Figure 6: Protocol of Ammonia concentration test

Source: Central Lab,DPHE

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
Tap	Coliform (Faecal)	0	24	N/100 ml	MFM
Tap	Coliform (Faecal)	0	12	N/100 ml	MFM
Filter	Coliform (Faecal)	0	36	N/100 ml	MFM
Filter	Coliform (Faecal)	0	12	N/100 ml	MFM
Tap	Coliform (Faecal)	0	4	N/100 ml	MFM
Tap	Coliform (Faecal)	0	12	N/100 ml	MFM
Tap	Coliform (Faecal)	0	8	N/100 ml	MFM
Tap	Coliform (Faecal)	0	184	N/100 ml	MFM
Filter	Coliform (Faecal)	0	72	N/100 ml	MFM
Tap	Coliform (Faecal)	0	24	N/100 ml	MFM
Tap	Coliform (Faecal)	0	8	N/100 ml	MFM
Filter	Coliform (Faecal)	0	6	N/100 ml	MFM
Filter	Coliform (Faecal)	0	124	N/100 ml	MFM
Filter	Coliform (Faecal)	0	84	N/100 ml	MFM
Filter	Coliform (Faecal)	0	8	N/100 ml	MFM

Sample Source	Water Quality Parameter	Bangladesh Standard	Concentration Present	Unit	Analysis Method
Filter	Coliform (Faecal)	0	24	N/100 ml	MFM
Filter	Coliform (Faecal)	0	92	N/100 ml	MFM
Tap	Coliform (Faecal)	0	68	N/100 ml	MFM
Tap	Coliform (Faecal)	0	140	N/100 ml	MFM
Filter	Coliform (Faecal)	0	6	N/100 ml	MFM

Through the information provided in Table 1, the samples are found to be contaminated by FC as revealed by fecal coliforms plate count indicating the presence of pathogenic bacteria. The result also shows the FC counts are far beyond by the limit set by WHO (World Health Organization) which is supposed to be zero per 100 ml of water. Three of the samples are at highly contaminated as the FC count is more than 100. Eleven samples are moderately risky as the FC count is 10-100 per 100 ml. No sample is found having 0 FC count i.e. no sample is found to be satisfying the standard of Bangladesh.

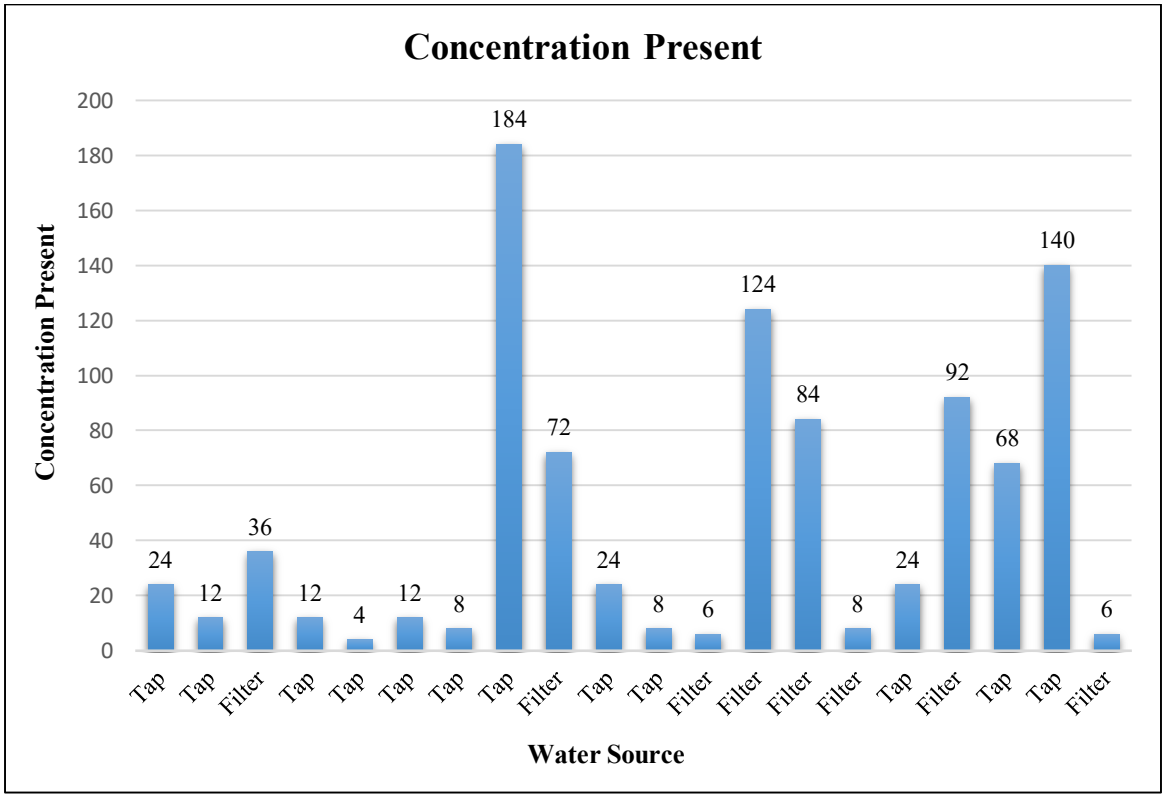

Figure 7: Bar chart showing FC count

Figure 7 shows the FC count result from the Central Lab, Mohakhali. The FC count having highest numbers is both tap and filter water samples. Two of them are tap water samples and one is filter water sample.

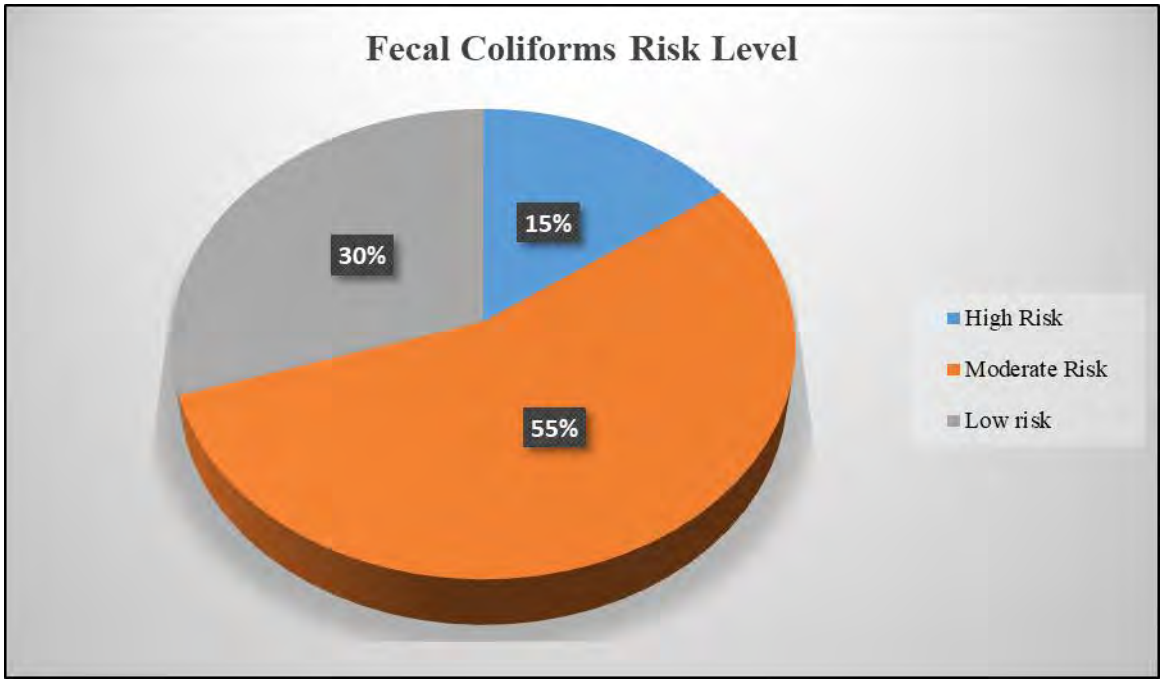

Figure 8: Risk Percentage of Fecal Coliforms from Central Lab results

In figure 8, FC risk level has been classified in percentage based on the result obtained from the central lab, Mohakhali. It can be seen that 55% of the total sample is moderately risky i.e. the FC count is from 10 to 100 in this class. Again, 30% of the samples are at low risk which means, the FC count in this class is below 10. However, 3 samples were found to be highly risky as the FC count of these 3 samples was found to be above 100 per 100 ml (as per the WHO standard classification of FC count).

Table 2: Result of Ammonia Concentration in the Samples by Central Lab

Sample source	Bangladesh Standard	Concentration Present	Unit	Analysis Method	LOQ
Tap	0.5	0.1	Mg/L	UVS	0.1
Tap	0.5	0.3	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1
Tap	0.5	0.3	Mg/L	UVS	0.1
Tap	0.5	0.2	Mg/L	UVS	0.1
Tap	0.5	0.2	Mg/L	UVS	0.1
Tap	0.5	0.3	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1
Tap	0.5	0.1	Mg/L	UVS	0.1
Tap	0.5	0.2	Mg/L	UVS	0.1
Filter	0.5	0.4	Mg/L	UVS	0.1
Filter	0.5	0.3	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1
Filter	0.5	0.2	Mg/L	UVS	0.1
Filter	0.5	0.3	Mg/L	UVS	0.1
Tap	0.5	0.2	Mg/L	UVS	0.1
Tap	0.5	0.3	Mg/L	UVS	0.1
Filter	0.5	0.1	Mg/L	UVS	0.1

As shown in Table 2, all the samples have ammonia concentration below 0.5 mg/L i.e. all the are found to be under the standard of Bangladesh.

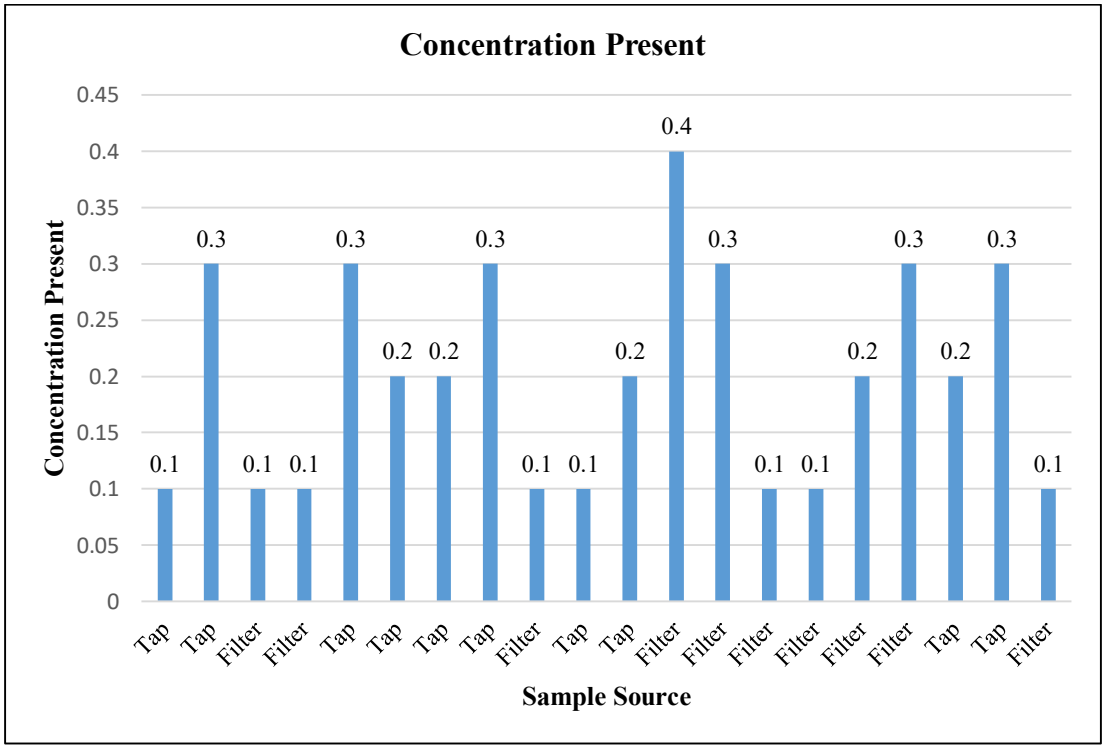

Figure 9: Bar chart showing ammonia concentration

As per Figure 9, 8 samples have ammonia concentration of 0.1 mg/L, 5 have 0.2 mg/L, 6 have 0.3 mg/L and 1 has 0.4 mg/L. If the concentration of ammonia is above 0.2 mg/L, bad odor and taste can be felt in water.

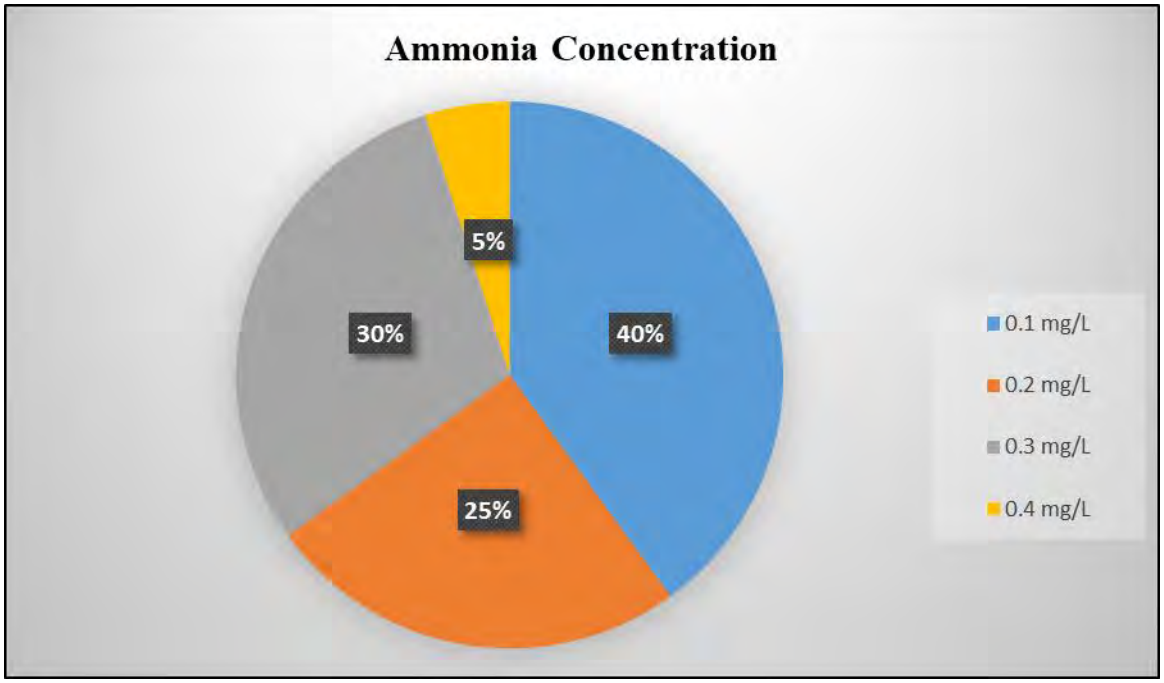

Figure 10: Percentage of ammonia concentration from Central Lab, Mohakhali

As per the standard of Bangladesh, the concentration of ammonia in drinking and household water is 0.5 mg/L. In figure 8 it can be seen that, all the samples satisfied the ammonia concentration i.e. all the samples have the concentration of ammonia below 0.5 mg/L.

Chapter 4

Discussion

The objective of this study was to analyze the microbial quality of the water samples collected from the restaurants and tea-stalls of Mohakhali area. Though the samples collected from tap have variation in FC count, the supplier is same for all the restaurants. There might be a possibility of contaminated pipes for which the FC count is shocking in some cases. Also leakage of pipes through which air can pass might cause contamination. Eleven samples are found to be moderately at risk, six are at low risk and three are at high risk. No restaurant or tea-stall was found to satisfy the WHO or Bangladesh standard of Fecal Coliform count. The lowest count of FC was 4/100ml and highest count was 184/100ml. A the most effective solution might be increasing awareness among the general mass and the restaurant owners for which regular inspection of pipelines can be possible. Also, the handling of water dispensers in the tea stalls and restaurants is very important. Due to careless handling of the dispensers of filters, contamination might increase in drinking water.

As it has been mention earlier, ammonia concentration level is related to FC count. If the concentration level is above the Bangladeshi standard, the possibility of FC contamination is very high. However, we have found the level of ammonia is within range but the FC count is unsatisfactory. One link has been found through the result, the samples having higher concentration of ammonia i.e. 0.2-0.3 mg/L, have higher FC count. Also to mention, two samples were collected from jugs which contained filter water and those two samples contained the highest FC count. Similar results were found through the research performed by M.Moniruzzaman in which the water samples collected from glasses had higher FC count than the samples collected from dispensers or filters.

Focusing on the mass exposed to the restaurants and tea-stalls, it has been found that not same class of people is being exposed to the same zone. The samples collected from behind of TB gate are found to be more contaminated than the samples collected from the premise of Brac University. The main customers of the restaurants behind TB gate are the attendants of the patients of TB Hospital Mohakhali. Also rickshaw pullers and drivers visit those road-side restaurants. Therefore, these people are at high to moderate risk levels. However, one of the restaurants behind Square Building has the highest count of FC. A lot of Brac University students visit that restaurant for both breakfast and lunch. Thus, for that particular restaurant the students visiting that restaurant are at high risk. However, the other restaurants around Brac University fall under moderate to low risk level as per the WHO standard guideline of household and drinking water.

Tea-stalls are found to be under moderate risk as the FC count is 10-100 per 100 ml. Service holders of different sectors including the staffs of Brac University, Brac Centre and Square Pharmaceuticals visit these tea-stalls very frequently. Thus they are at a high risk since they visit the stalls multiple times per day.

One thing was noted during the survey, the water dispensers in filters were uncovered and were handled carelessly in all of the restaurants. The owners might not use the water dispensers from the companies which supply water dispensers. It could not be figured out whether the dispensers were filled with tap water or collected from the dispenser suppliers.

Chapter 5

Conclusion

The importance of pure water is indescribable in our lives. Not only humans, even animals need fresh water for living. Due to improper sewage system and industrial wastes along with man-made pollution, the level of fresh water from the ground is decreasing day by day. It has become a must for us to stop this pollution as it is not only harming the environment, but also our health. The future generations' lives are at stake if pure and fresh water is not available. The tap water of a lot of developed countries are fresh enough to drink without even boiling. The reason behind contamination in the pipe water supplied by WASA needs to be figured out as the Central Lab, Mohakhali has showed huge differences in the result of FC test in case of a few restaurants. Also, the difference in the level of ammonia concentration needs to be highlighted as it is the major reason of higher FC count.

Chapter 6

Future Work

The identification of the microorganisms during the antibiotic resistance test is necessary. The water samples were store at -30 degree Celsius before culturing of bacteria. At this temperature bacteria, is not supposed to survive. Thus it is very important to figure out the microorganisms. Also, the sites from where highest FC counts have been obtained needs to be checked again.

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