Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Jatrabari, Bangshal, Khilgaon, and Wari

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May 2019

Declaration

I hereby declare that the thesis project titled "Fecal organisms in supplied and domestically treated waters in Dhaka: insights from Bangshal, Khilgaon, Jatrabari and Wari" has been written and submitted by me, Saiful Islam Shanto and has been carried out under the supervision of Mahbubul Hasan Siddiqee, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Acknowledgement

First and foremost, I would like to express my thanks to Almighty Allah because He has given me the opportunity and strength to finish this research. I am also thankful for His blessings to my daily life, good health and healthy mind. I acknowledge my esteem to Professor **A F M Yusuf Haider** and Chairperson of MNS Department and Professor **Mahboob Hossain** for allowing me and encouraging me to complete my undergraduate thesis.

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

I am also grateful to **Md. Mahmudul Hasan** for his great support. I am extremely thankful and indebted to them for sharing expertise, and sincere and valuable guidance and encouragement extended to me.

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

Saiful Islam Shanto

May, 2019.

Abstract

Dhaka, the capital city of Bangladesh, is the most densely populated city of the world. So, it is always a challenge to meet the ever increasing demand for pure drinking water of this huge population. This study aims to detect the presence of fecal coliform (FC) and *Salmonella* spp. in the supplied and domestically treated waters in Bangshal, Khilgaon, Jatrabari and Wari. A total of 110 samples were collected from October 2018 to April 2019. The average fecal coliform count in raw water for Jatrabari zone was 58.33, for Wari 10.17, for Khilgaon 49.83 and for Bangshal 17.86; in boil water 4.25 for Jatrabari, 0.00 for Wari, 4.38 for Khilgaon, for 0.00 Bangshal; in filtered water 1.74 for Jatrabari, 0.00 for Wari, 1.04 for Khilgaon, for 0.00 Bangshal. PCR-based detection of *Salmonella* spp. suggests occasional contamination in water samples from Bangshal, Khilgaon, Jatrabari and Wari along with Korail Slum and Tea stalls sample. From this study reveals possibility of transmission of fecal pathogens via drinking water and generates evidence that contaminated drinking water is an overlooked health hazard in Bangshal, Khilgaon, Jatrabari and Wari.

Contents

Chapter 1

Introduction

1.1 Importance of water

Water is significant in light of the fact that it is basic to life on earth. People can just live three days without water; however, it is conceivable to live a long time without nourishment. Water is particularly significant for human wellbeing, medication, farming and industry. The human body is made up for the most part of water. Eighty-five percent of the mind is made of water, while blood comprises of 80 percent water. Water is fundamental for almost every real capacity, including assimilation and disposal. Water transports supplements from sustenance into the circulatory system, at that point on to the cells in the body. Water is related with each generous limit from processing and course through to the control of body temperature and the release of waste things. The water in our bodies is always being used or lost from the body. Some is used or devoured by the limits it performs and some is lost through sweat, pee and defecation. Since the water in our bodies is perpetually being used or lost, it ought to be continually replaced, and the best fluid to override it with is water. Hence, the water that we are drinking must be unadulterated. Generally, we drink water by bubbling or separating it. Household water use includes utilization of water, nourishment arrangement, washing, washing garments and dishes, brushing teeth, watering the yard and patio nursery and so on. Presently the inquiry comes, the water that we use for our day by day work and drinking whether it is unadulterated to utilize or not.

1.2 Possibility of fecal contamination

As a rule, expanded dimensions of fecal coliforms give a notice of disappointment in water treatment, a break in the honesty of the conveyance framework, conceivable defilement with pathogens. At the point when levels are high there might be a raised danger of waterborne gastroenteritis. Abnormal state of sullying in drinking water has been represented a few

examinations however issue remains unaltered. Malady causing living beings (pathogens) transmitted by methods for drinking water are dominatingly of fecal source. In spite of the way that, the immediate detachment of intestinal pathogens is unfeasible; rather general wellbeing investigators decide the quantity of marker microorganisms. Visit examination of fecal marker life forms remain the most effortless strategy for assessing the sanitation states of water. The perfect fecal pointer ought to fulfill the majority of the particular criteria, for instance, reliably nearness in the defecation, powerlessness to duplicate outside the intestinal tract, is at solid relationship with the nearness of pathogenic microorganisms, and grant straightforward research facility strategy (Savichtcheva and Okabe, 2006).Marker living beings of fecal contamination incorporate the coliform bunch all in all and particularly *Escherichia coli.*

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

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

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

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

1.3. Possibilities of infection via drinking water

Among 50 maladies normal in Bangladesh, 40 of them including looseness of the bowels, diarrhea, typhoid, parasitic worm disease so on are identified with the tainted sustenance and water. Various strains of *E. coli* in drinking water are in charge of an assortment of sicknesses including looseness of the bowels, diarrhea, hemolytic uremia disorder (kidney disappointment), bladder diseases, septicemia, pneumonia, meningitis (Acharjee et al., 2011). Shiga poisons (Stx) which is known as verotoxins (Vtx), includes two noteworthy subtypes, shiga poison 1 *(Stx1*) and shiga poison 2 (*Stx2*) are delivered by a few enteric pathogens, *Shigelladysenteriae* (serotype 1 just) and enterohaemorrhagic *Escherichia coli* (EHEC). They are facultative anaerobic which can mature sugars with the creation of natural corrosive and gas. These three genera on a sort of maturation called "blended corrosive aging," anyway differentiate in different physiological qualities. More than 45,000 under-five youngsters bite the dust each year in Bangladesh from looseness of the bowels brought about by defiled water, says a report of World Wellbeing Association.

In any case, the World Wellbeing Association (WHO) assesses that about 1.1 billion people comprehensively drink hazardous water (Kindhauser, 2003) and most by a wide margin of diarrheal sickness on the planet (88%) is owing to risky water. In addition, social and ecological changes continue achieving new or re-rising waterborne pathogen issues. For example, environmental change was surveyed to be mindful in 2000 for roughly 2.4% of overall loose bowels, 6% of intestinal sickness in some center pay nations and 7% of dengue fever in some industrialized countries. By and large, the inferable mortality was 154 000 (0.3%) passing's and the inferable weight was 5.5 million (0.4%) (Ashbolt, 2004). Environmental change impelled flooding and dry seasons can affect family water and sanitation foundation and related wellbeing dangers. For example, cholera also, water inadequacies because of dry spell can expand dangers of diarrheal sickness.

1.4. Water borne disease in Dhaka

Waterborne illness any malady that is transmitted or spread through debased water. In Dhaka individuals are bothered with water borne infections through drinking water just strengthens the condition. Despite legislative plans and activities, non-administrative exercises or individual mindfulness, the issue of safe water access just as inescapability of water borne illnesses are still very common.(Islam et al., 2001). Various factors might be incorporated for pollution of drinking water. Ground water can end up debased from normal sources or different sorts of human activities. Private, common, business, mechanical, and agrarian exercises would all have the option to impact ground water quality. Sullying of cylinder well water appears to be related to different factors, including closeness of toilets or channels to the cylinder wells, tube well profundity or strategy for finishing, and factors, for example, the act of cylinder well preparing may likewise be included. Provided water may be sullied through the dispersal pipes because of

spillage. It tends to be endorsing avoiding arrivals of wastewater without treatment, chiefly from septic tanks, which are widely utilized in the territory (Parvez, Liza, and Marzan, 2016). Diarrheal ailment is an essential reason of dreariness and mortality in creating nations, including Bangladesh. Among 50 maladies regular in Bangladesh, 40 of them including loose bowels, looseness of the bowels, typhoid, and parasitic worm disease so on are identified with the polluted water (MdShahidul, Mehadee, and Sunjukta, 2014). As youngsters are the most in danger gathering, it is essential to see what sorts of pathogens result in their looseness of the bowels. Concentrates in Dhaka, Bangladesh have exhibited that in the stools some 75% of diarrheal youngsters and 44% of control kids have an enteric pathogen. (Ashbolt, 2004) The primary life forms related with looseness of the bowels being rotavirus, Cryptosporidium parvum and the accompanying bacterial pathogens: *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* [ETEC], enteropathogenic *E.coli* [EPEC], *Shigella spp*. furthermore, *Vibrio cholerae*O1 or O139 and to a lesser degree *Bacteroidesfragilis* and *Clostridium difficile* (Albert et al., 1999). In any of the youngsters in the Dhaka considers, some other potential bacterial pathogens, *Plesiomonasshigelloides*, *Salmonella spp.* diffusely follower *E. coli*. Alongside the parasitic protozoa *Entamoebahistolytica* and *Giardia lamblia*were not fundamentally connected with looseness of the bowels and *enteroinvasive E. coli*, enterohemorrhagic *E. coli* [EHEC] and *Cyclosporacayetanensis* were not recognized. (Ashbolt, 2004).

1.5. Selected zones of this study (Jatrabari, Bangshal, Khilgaon and Wari)

Dhaka is the biggest and most populated city in Bangladesh. In Bangladesh as well as it is a standout amongst the most populated urban communities on the planet. The all-out zone of Dhaka city is 300 square kilometers where 18.237 million of individuals are living. It is a

standout amongst the most thickly populated zones on the planet, with a thickness of 23,234 individuals for every square kilometer. Dhaka city is isolated into various zones.

Jatrabari, Bangshal, Khilgaon, Wari are the four metropolitan and neighborhoods in Dhaka city. Absolute territory of Jatrabari region is 13.19 km2 where 260772 individuals are living, populace thickness of this zone is 19770/km^2. Another presumed territory of Dhaka city is Khilgaon zone. All out territory of Khilgaon zone is 14.02 km^2 where 230902 individuals are living. Populace thickness of this region is 16,000/km^2.From that point forward; All out zone of Wari region is 2.27 km^2 where 1050 individuals are living. Populace thickness of this zone is 462/km^2. That implies, the populace thickness of Jatrabari territory is more than both the Khilgaon, and Wari.

1.6. Aim of the study

This paper gives a concise survey of microbiological nature of faucet water provided by DWASA and locally treated water in Dhaka city by virtue of its essentialness in general wellbeing. This investigation will make mindfulness among the populace living in the chose zones. The particular target of this examination is to decide the absolute burden and dimensions of pollution, thermo tolerant *E.coli* were quantities in the water tests tried and to recognize *Salmonella spp.,* the noticeable living being for typhoid fever. As the ordinary procedures subject to bacterial culture and serological tests take couple of days accordingly atomic strategies dependent on genotype of the microscopic organisms are picked over customary strategy in this investigation to recognize *Salmonella spp.*

Chapter: 2

Materials and Method

2.1. Sampling sites:

A total of 110 water samples from Jatrabari, Wari, Bangshal and Khilgaon.

Figure 2.1.1: Satellite view of Jatrabari sampling zone

Figure 2.1.1: Satellite view of khilgaon sampling zone

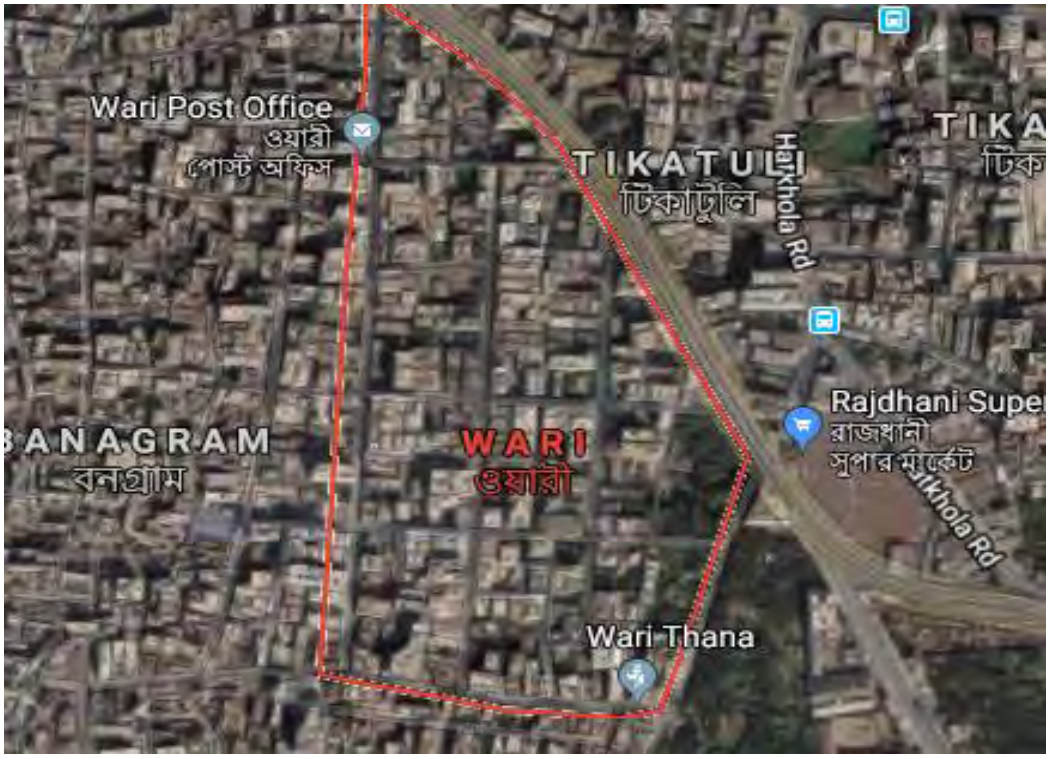


Figure 2.1.1: Satellite view of Wari sampling zone

Figure 2.1.1: Satellite view of Bangshal sampling zone

2.2. Sample collection

Water tests were month to month gathered from October 2018 to April 2019. Tests were gathered from various locales of three focused on zones (Jatrabari, Wari, Bongshal and Khilgaon). For test gathering, the accumulation bottles were recently autoclaved.

2.3. Sample processing

2.3.1. Filtration

By utilizing film channel papers, 100ml of gathered examples were sifted and put on m-FC agar plates and the plates were brooded at 44.5℃ for 24 hours. Following 24 hours, the plates were watched. Provinces made by fecal coliforms can appear as factor shades of blue-hued settlements on the layer channel. The settlements were affirmed by sub refined them on EMB agar. Just the settlements that had showed up as metallic green sheen are *E. coli*. Same gathered examples about 100ml of were separated once more. Film channel papers were set in cone shaped flagons containing 50ml of TSB enhanced with 2.5% NaCl and the carafes were brooded at 37◦C for 12 hours. 300µl of autoclaved glycerol were set in autoclaved miniaturized scale rotator tubes. After brooding was finished, TSB were exchanged to miniaturized scale rotator tubes and vortexed. Miniaturized scale rotator tubes were put away at - 20◦C. 1.5ml of brooded TSB were exchanged to an autoclaved smaller scale rotator tubes and centrifuged at 14,000 rpm for 10 minutes. After centrifugation the supernatants were disposed of. The pellets were put away at - 20℃ after the mouth of the smaller scale axis tubes had been wrapped with parafilm for DNA extraction.

2.3.2. DNA extraction protocol

A few microorganisms require the execution of explicit strides for genomic investigation, for example, in cell DNA extraction. It is realized that basically heating up a suspension of *E.coli* or

Salmonella spp, for example, is a compelling strategy for instigating cell lysis, for doing Polymerase Chain Responses (PCR) (Ranjbar, Naghoni, Afshar, Nikkhahi, and Mohammadi, 2016).

Following 12 hours of hatching 1.7ml of TSB were exchanged to a 2ml small scale axis tubes and loaded with 0.3ml of glycerol. TSB containing microorganisms were exchanged to miniaturized scale rotator tubes about 1.5ml and that centrifuged for 10 minutes at 14,000 rpm (Kobayashi et al., 2009). The pellet were gathered and included 400µl of refined water that were put away at room temperature a modified the blend for washing. The blends were centrifuged for 5 minutes at 13,000 rpm and after that expelled supernatant. At that point the pellets were re-suspended with 400µl of refined water. The cells were lysed at 100◦C for 7 minutes. After warmth stuns were performed smaller scale axis tubes were moved in ice for 10 moments to performed cold stun. Following 10 minutes, axes were performed for 5 minutes at 13,000 rpm. The supernatant contained with DNA were moved into new smaller scale rotators tubes and wrapped with parafilm and put away at - 20◦C.

2.4. Raw DNA gel run

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

Table 2.4 Sequences of primers used for amplification by PCR.

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

2.5. Preparation of control for PCR

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

2.6. PCR

PCR test were performed in cylinders with an all-out volume of 20µl. The response blends usually contained nuclease free water 4µl, forward groundwork 2µl, turn around preliminary 2µl, layout 2µl. What's more, ace blend 10µl. Pipetting was done in cautious way with the goal that

no air pockets present and perform turning. After the underlying planning was taken, PCR were performed under the accompanying conditions: 35 cycles with beginning denaturation at 94˚C for 5 minutes warmth denaturation at 95°C for 30 seconds, preliminary tempering at 60°C for 30 seconds, and DNA augmentation at 72°C for 60 seconds and last expansion at 72˚C for 8 minutes in small scale axis tubes angle ace cycler. Sterile water was utilized rather than layout DNA to give a negative control to screen the tainting of outer DNA in the PCR reagents in PCR response.

2.7. Gel electrophoresis

Customary agarose gel electrophoresis was performed to affirm that the PCR response enhanced the right target quality. The enhanced DNA were isolated by 1% agarose gel electrophoresis at 70 voltages, recolored with ethidium bromide, and envisioned by UV transilluminator. 1500 base-pair of DNA stepping stool was utilized.

Chapter: 3

Results

3.1. Fecal coliform counts

Fecal coliforms are the creatures used to show the nearness of fecal tainting and to screen the expulsion of pathogens from wastewater treatment plants. The recognition of *E. coli* gives unmistakable confirmation of fecal contamination. Ordinarily expanded dimensions of fecal coliforms give a notice of carelessness in water treatment, a break in the uprightness of the dispersion framework, possible contamination with pathogens. Exactly when levels are high there may be a raised threat of waterborne gastroenteritis. The worthy element of *E. coli* is dictated by hazard investigation subject to measurements to ensure human wellbeing. Drinking water should have no *E. coli* after treatment.

Table 3.1 Fecal coliform counts in Jatrabari Zone

Jatrabari						
Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	5.50(n=4)	NA	1.25(n=4)	3.32	0.00	0.23
November	27.75(n=4)	8.00(n=4)	3.00(n=4)	14.82	4.32	0.52
December	31.00(n=4)	2.00(n=4)	2.00(n=4)	15.64	0.82	0.66
January	82.75(n=4)	15.50(n=4)	4.00(n=4)	25.75	8.50	0.82
March	103.00(n=2)	NA	NA	38.89	0.00	0.00
April	100.00(n=2)	NA	NA	35.36	0.00	0.00
Average	58.33	4.25	1.71			

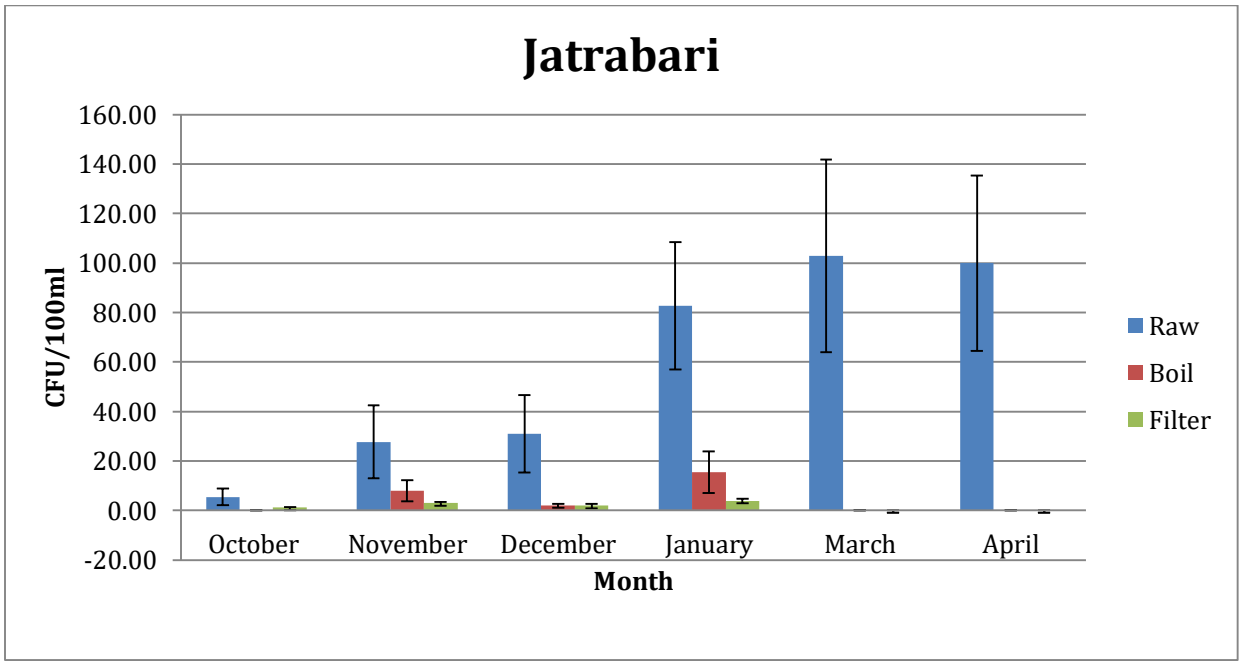


Figure 3.1: Fecal coliform counts in raw, boil and filtered water

3.1.1. Monthly variation:

A total of 31 samples were collected from Jatrabari zone. In October, the average count for fecal coliform in raw water was 5.5, in boil water NA and in filtered water 1.25. In November the average count for fecal coliform in raw water was 27.75, in boil water 8 and in filtered water 3; in December the average count for fecal coliform in raw water was 31, in boil water 2 and in filtered water 2; in January the average count for fecal coliform in raw water was 82.75, in boiled water 15.5 and in filtered water 4. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count for fecal coliform in raw water was 103, in boiled water NA and in filtered water NA. In April, fecal coliform count was 100 in raw water, NA in boiled water and NA in filtered water. In case of raw water, the fecal coliform count has been increased only from March to April has been decreased that is 100 from 103. In raw water, from December to January fecal coliform counts

has been increased highly from 31 to 82.75. In case of boiled water, in October and March we have not get any reading other than these, fecal coliform reading in November 8, December 2, January 15.5. The Fecal count for the boiled water always fluctuates from month to month. For the Filtered water, fecal coliform reading was NA in April and other than this, in October 1.25, November 3, December 2, January 4.

3.1.2. Standard deviation for raw, boil and filtered water:

Standard deviation for raw water from October to April, except February were (3.32, 14.82, 15.64, 25.75, 38.89, 35.36) for boil water samples (0, 4.32, 0.82, 8.50, 0, 0) and for filtered water samples (0.23, 0.52, 0.66, 0.82, 0, 0) As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more spread out or dispersed than filtered and boil water samples data.

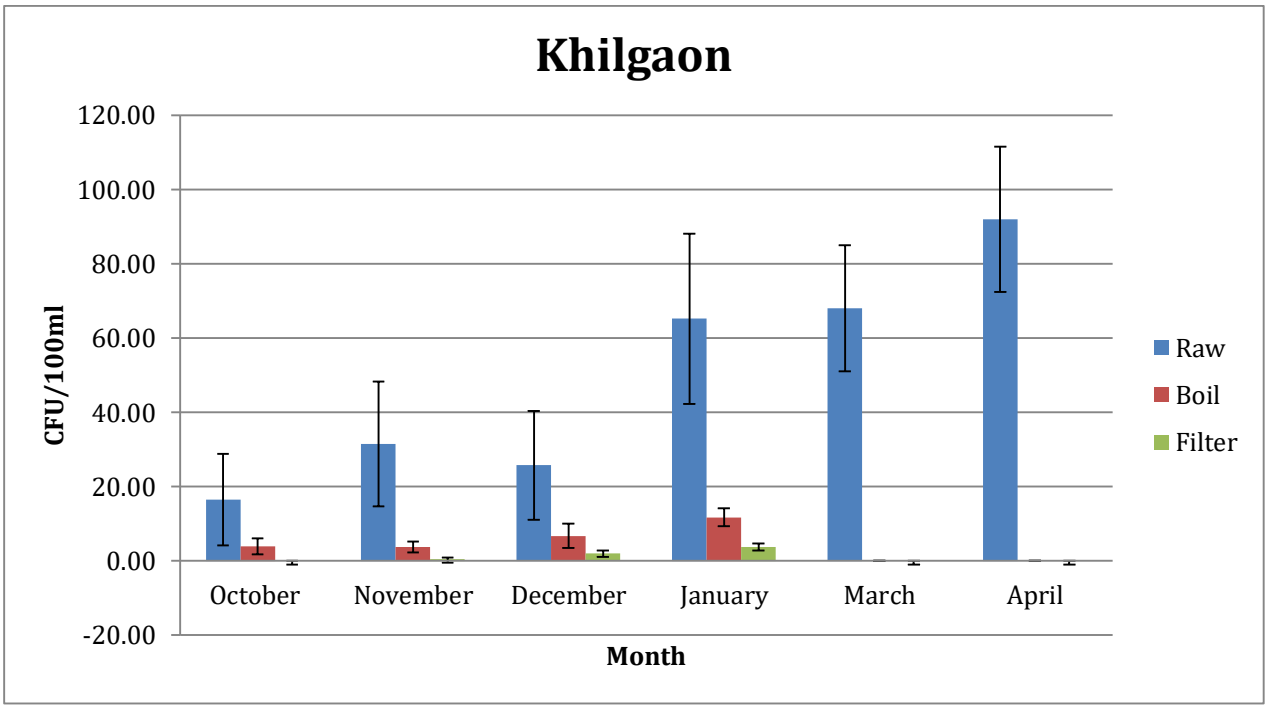
3.1.3. Seasonal variation:

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

Table 3.2: Fecal coliform counts in raw, boil and filtered water in Khilgaon Zone

khilgaon						
Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	16.50(n=4)	4.00(n=4)	NA	12.37	2.16	0.00
November	31.50(n=4)	3.75(n=4)	0.50(n=4)	16.78	1.45	0.42
December	25.75(n=4)	6.75(n=4)	2.00(n=4)	14.64	3.23	0.86
January	65.25(n=4)	11.75(n=4)	3.75(n=4)	22.90	2.36	0.92
March	68.00(n=2)	NA	NA	16.97	0.00	0.00

April	92.00(n=2)	NA	NA	19.55	0.00	0.00
Average	49.83	4.38	1.04			



3.2: Fecal coliform counts in raw, boil and filtered water in Khilgaon Zone

3.2. Monthly Variation:

A total of 28 samples were collected from Khilgaon zone. In October, the average count for fecal coliform in raw water was 16.5, in boil water 4 and in filtered water NA. In November the average count for fecal coliform in raw water was 31.5, in boil water 3.75 and in filtered water 0.5; in December the average count for fecal coliform in raw water was 25.75, in boil water 6.75 and in filtered water 2; in January the average count for fecal coliform in raw water was 65.25, in boiled water 11.75 and in filtered water 3.75. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count

for fecal coliform in raw water was 68, in boiled water NA and in filtered water NA. In April, fecal coliform count was 92 in raw water, NA in boiled water and NA in filtered water. In case of raw water, the fecal coliform count has been increased from October to April has been increased that is 16.5 to 92 except only November to December 31.5 to 25.75. In raw water, from December to January fecal coliform counts has been increased highly from 31 to 82.75. In case of boiled water, fecal coliform reading in October 4, November 3.75, December 6.75, January 11.75. The Fecal count for the boiled water always fluctuates from month to month. For the Filtered water, fecal coliform reading was NA in October, April and March and other than these, November 0.5, December 2, January 3.75.

3.2.1. Standard deviation for raw, boil and filtered water:

Standard deviation for raw water sample from October to April, except February were (12.37, 16.78, 14.64, 22.90, 16.97, 19.55), for boiled water (2.16, 1.45, 3.23, 2.36, 0, 0) and for filtered water samples (0, 0.42, 0.86, 0.92, 0, 0). As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more dispersed than filtered water samples data and boiled water sample.

3.2.2. Seasonal variation*:*

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

Table 3.3 Fecal coliform counts in raw, boil and filtered water in Wari.

WARI						
Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	0.00(n=2)	0.00(n=2)	NA	0.00	0.00	0.00
November	20.00(n=2)	0.00(n=2)	0.00(n=2)	16.96	0.00	0.00
December	21.00(n=2)	0.00(n=2)	1.00(n=2)	19.80	0.00	0.71
January	0.00(n=2)	0.00(n=2)	0.00(n=2)	0.00	0.00	0.00
March	7.50(n=2)	NA	NA	3.54	0.00	0.00
April	12.50(n=2)	NA	NA	8.84	0.00	0.00
Average	10.17	0.00	0.17			

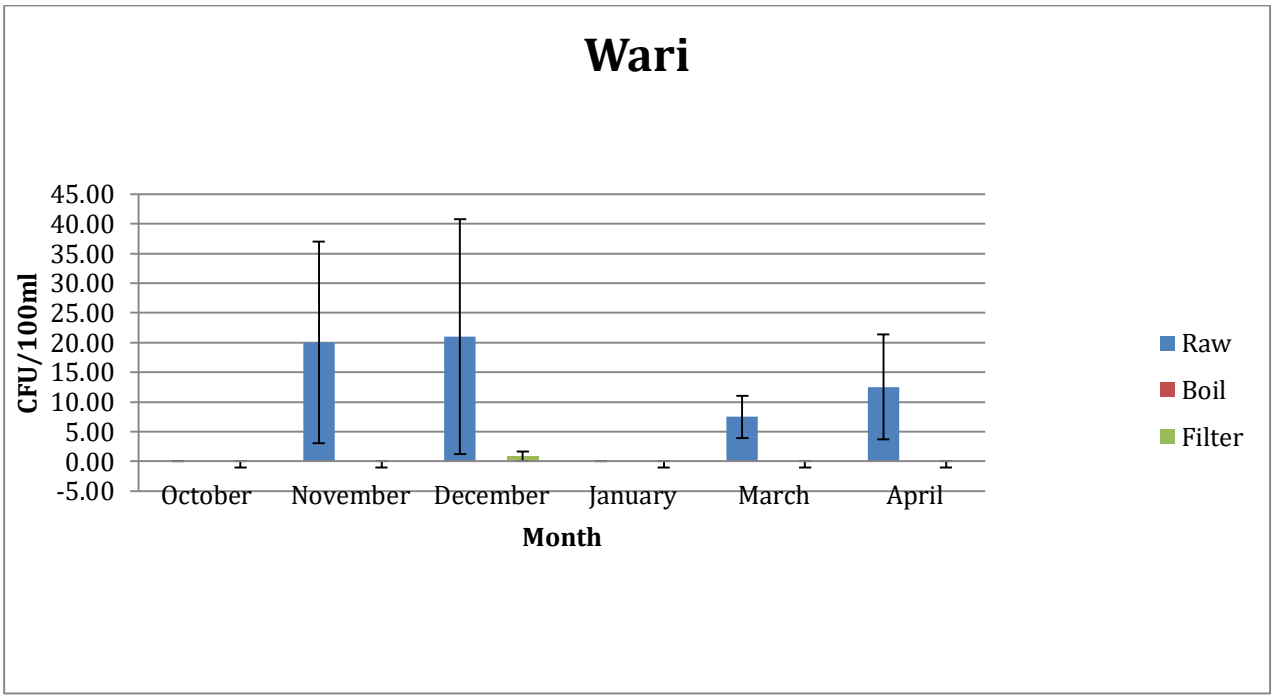


Figure 3.3 Fecal coliform counts in raw, boil and filtered water in Wari.

3.3. Monthly variation:

A total of 22 samples were collected from Wari zone. In October, the average count for fecal coliform in raw water was 0, in boil water 0 and in filtered water NA. In November the average count for fecal coliform in raw water was 20, in boil water 0 and in filtered water 0; in December the average count for fecal coliform in raw water was 21, in boil water NA and in filtered water 1; in January the average count for fecal coliform in raw water was 0, in boiled water 0 and in filtered water 0. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count for fecal coliform in raw water was 7.5, in boiled water NA and in filtered water NA. In April, fecal coliform count was 12.5 in raw water, NA in boiled water and NA in filtered water. In case of raw water, the fecal coliform count has been increased from October to April that is 0 to 12.5 except only December to January. In raw water, from December to January fecal coliform counts has been decreased from 21 to 0. In case of boiled water, fecal coliform reading in October 0, November 0, December 0, January 0, March has no reading then in April 0. For the Filtered water, fecal coliform reading was NA in October, March and April and other than these, November 0, December 1, January 0.

3.3.1. Standard deviation for raw, boil and filtered water:

Standard deviations for raw water sample from October to April, except February were (0, 16.96, 19.80, 0, 3.54, 8.84), for boil water samples are 0 and for filtered water samples are 0 except December 0.71. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more scattered than filtered and boil water samples data.

3.3.2. Seasonal variation:

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

Table 3.4: Fecal coliform counts in raw, boil and filtered water in Bangshal

Bangshal						
Month	Raw	Boil	Filter	SD Raw	SD Boil	SD Filter
October	10.67(n=3)	0.00(n=3)	0.00(n=3)	5.03	0.00	0.00
November	4.00(n=3)	NA	0.00(n=3)	3.06	0.00	0.00
December	2.50(n=3)	0.00(n=3)	NA	1.53	0.00	0.00
January	4.00(n=3)	0.00(n=3)	0.00(n=3)	3.61	0.00	0.00
March	32.00(n=2)	NA	NA	11.31	0.00	0.00
April	54.00(n=2)	NA	NA	16.97	0.00	0.00
Average	17.86	0.00	0.00			

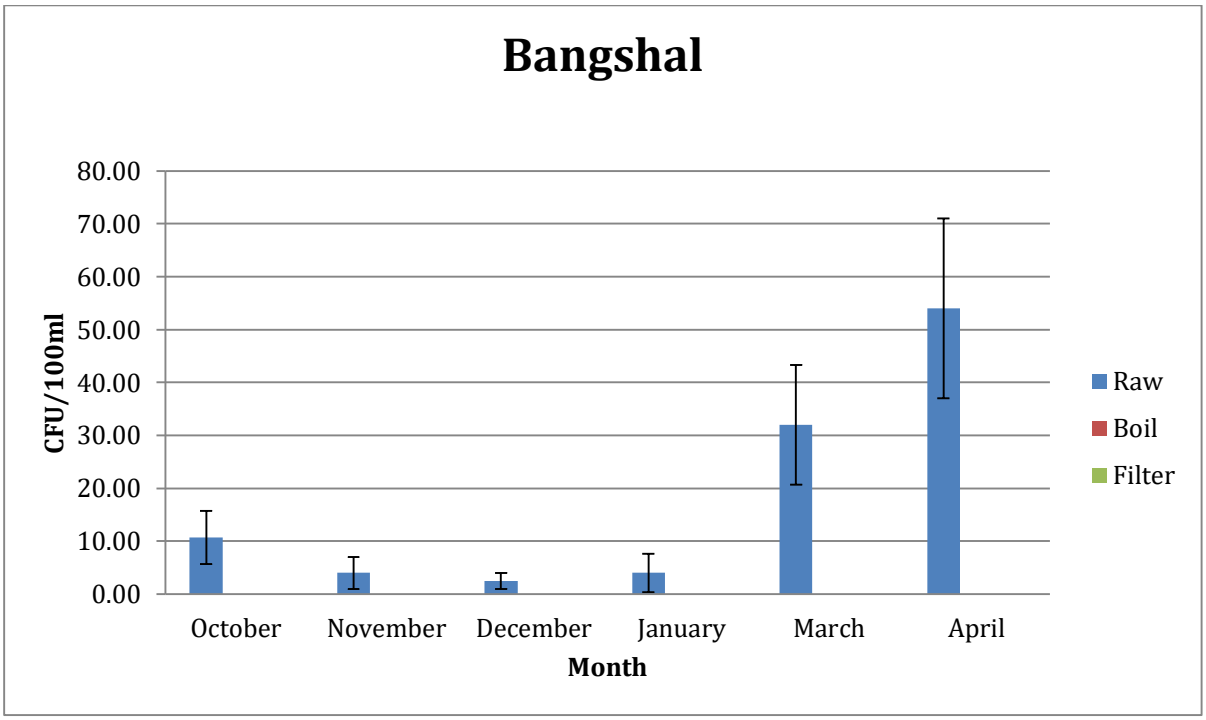


Figure 3.4: Fecal coliform counts in raw, boil and filtered water in Bangshal

3.4. Monthly variation:

A total of 20 samples were collected from Bangshal zone. In October, the average count for fecal coliform in raw water was 10.67, in boil water 0 and in filtered water 0. In November the average count for fecal coliform in raw water was 4, in boil water NA and in filtered water 0; in December the average count for fecal coliform in raw water was 2.5, in boil water 0 and in filtered water NA; in January the average count for fecal coliform in raw water was 4, in boiled water 0 and in filtered water 0. No sampling had been performed in February because volunteers for providing water samples were not available. In March, the average count for fecal coliform in raw water was 32, in boiled water NA and in filtered water NA. In April, fecal coliform count was 54 in raw water, NA in boiled water and NA in filtered water. In case of raw water, the fecal coliform count has been increased from October to April that is 10.67 to 54 except only

November to January. In case of boiled water, fecal coliform reading in October 0, November NA, December 0, January 0, March NA then in April NA.

3.4.1. Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples were from October to April, except February were (5.03, 3.06, 1.53, 3.61, 11.31, 16.97), for boil water samples are 0 and for filtered water samples 0. As the raw water samples had a higher standard deviation it could be said that the raw water samples data is more scattered than filtered and boil water samples data.

3.4.2Seasonal variation:.

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

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

Slum(korail)				
Month	Raw	Boil	Filter	SD Raw
October	340.00(n=3)	NA	NA	196.30
November	359.00(n=3)	NA	NA	207.27
December	412.00(n=3)	NA	NA	237.87
January	310.00(n=3)	NA	NA	178.98
Average	355.25			

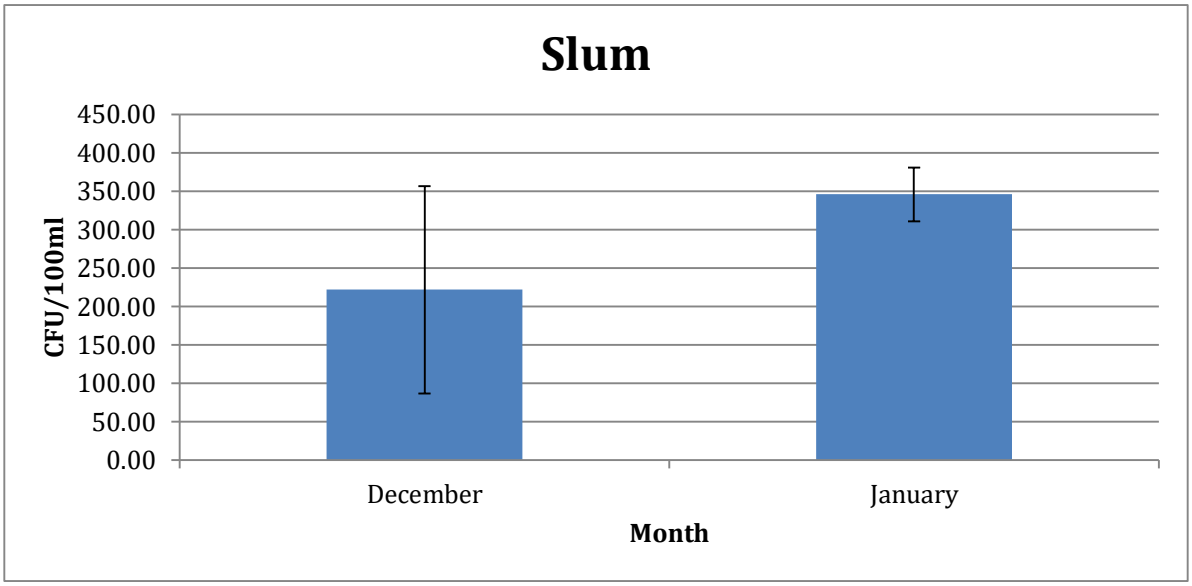


Figure 3.5 Fecal coliform counts in raw, boil and filtered water in Slum (korail)

3.5. Monthly variation:

A total of 5 samples were collected from Slum (korail) zone. Only we have detected the fecal coliform count for raw water that is in October 340, in November 359, in December 412, and in January 310. For Boiled and Filtered water, we have no value.

3.5.1. Standard deviation for raw, boil and filtered water:

Standard deviation for raw water samples from October to January were (196.30, 207.27, 237.87, 178.98). Only this is available so we cannot make any comparison.

3.5.2. Seasonal variation:

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

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

Tea Stalls				
Month	Raw	Boil	Filter	SD Boil
December	0.00	0.00	120.00(n=2)	7.07
January	0.00	0.00	60.00(n=2)	53.74
Average	0.00	0.00	90.00	

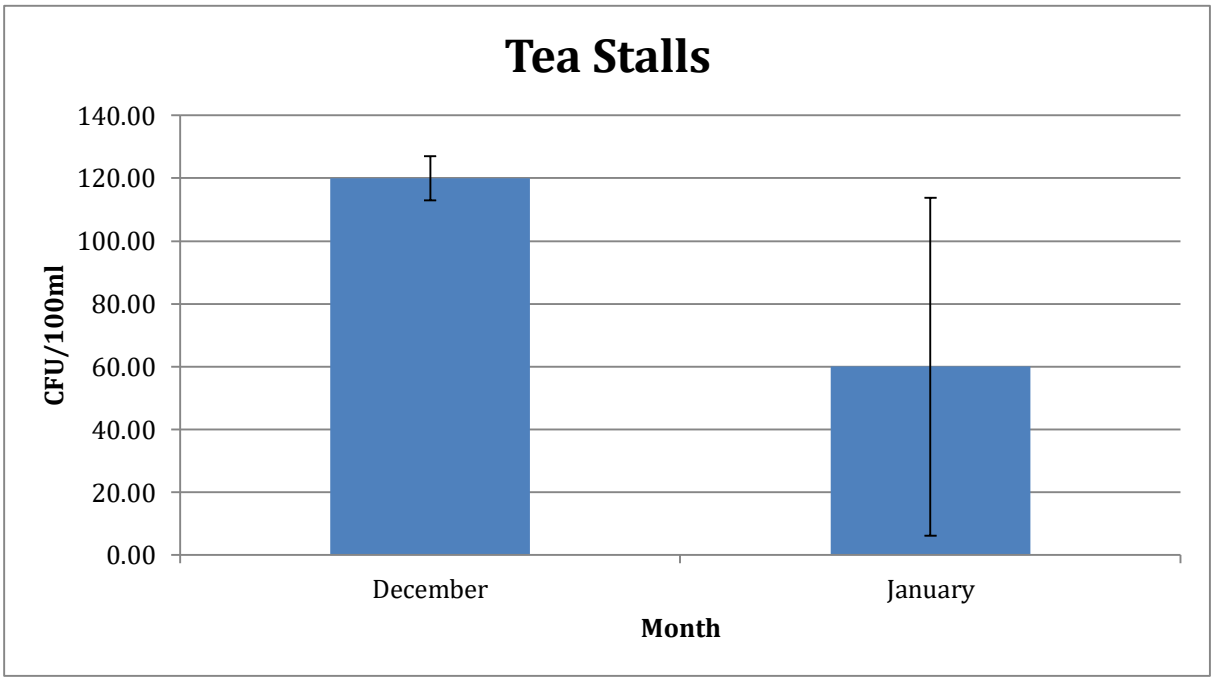


Figure 3.6 Fecal coliform counts in raw, boil and filtered water in Tea Stalls

3.6. Monthly variation:

A total of 4 samples were collected from Tea Stalls. The experiment has been performed only on the filtered water and the fecal coliform counts were 120 in December and 60 in January.

3.6.1. Standard deviation for raw, boil and filtered water:

Standard deviation for Filtered water samples in December 7.07 and January 53.74. There is no space to make any comparison due to shortage of data.

3.6.2. Seasonal variation:

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

3.7. Comparison

The average fecal coliform count in raw water for Jatrabari zone was 58.33, for Wari 10.17, for Khilgaon 49.83 and for Bangshal 17.86; in boil water 4.25 for Jatrabari, 0.00 for Wari, 4.38 for Khilgaon, for 0.00 Bangshal; in filtered water 1.74 for Jatrabari, 0.00 for Wari, 1.04 for Khilgaon, for 0.00 Bangshal From the bar chart, it clearly seen that Jatrabari zone contained the most fecal coliform counts followed by Khilgaon, Wari and Bangshal for raw, boil and filtered water.

3.8. PCR result:

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

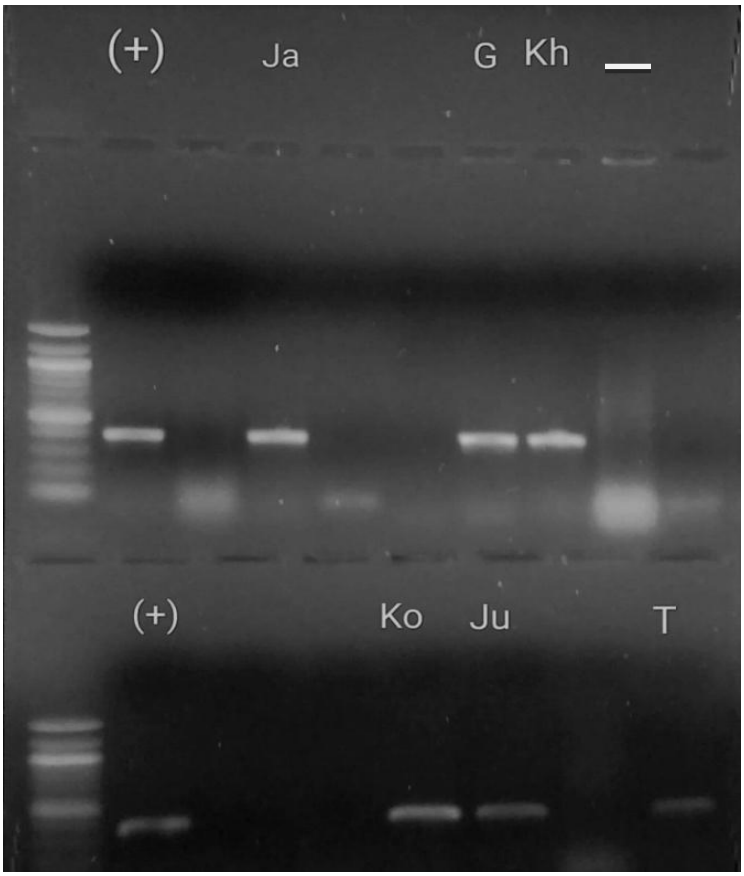


Figure 3.8.1.: The PCR results. Lanes Ja represented strains *Salmonella spp.* in Jatrabari zone, Kh represented strains *Salmonella spp.* in Khilgaon zone, G represented strains Salmonella spp. In Gandaria zone, ko represented strains *Salmonella spp.* in Korail Slum zone, Ju represented strains *Salmonella spp*. in Jurain zone, T represented strains *Salmonella spp*. in tea stall (Tejgaon) zone. L represented ladder which was 1500 base pair.

The reference strain *Salmonella spp.* used as positive control and *Shigella dysenteriae* as negative control. The six positive bands came from the samples that had fecal coliform count in above 300.

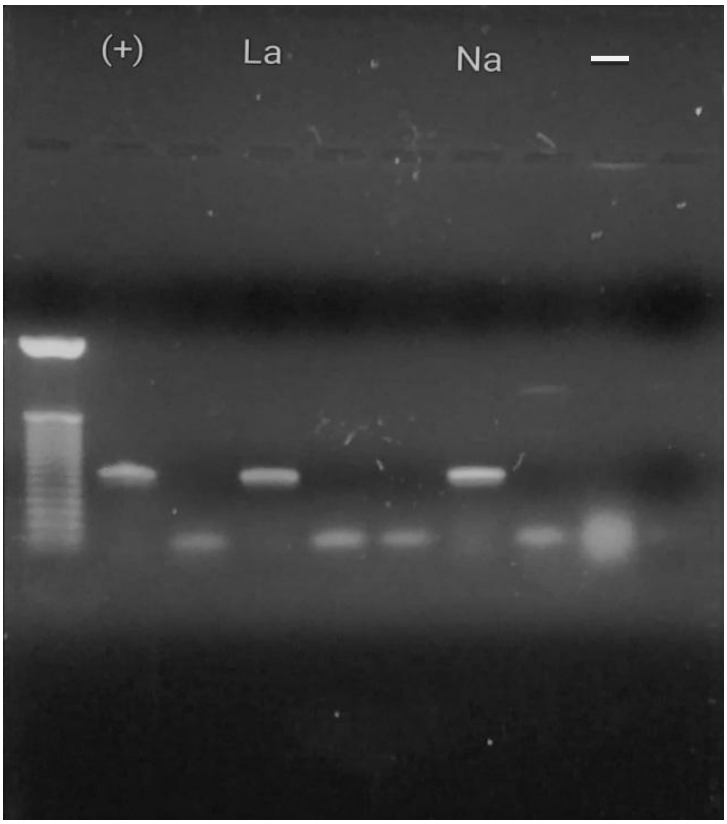


Figure 3.8.2: The PCR results. Lanes La represented strains *Salmonella spp*. in Lalmatia zone and Na represented strains *Salmonella spp*. in Narayanganj zone. The reference strain *Salmonella spp.* used as positive control. The two positive bands came from the samples that had fecal coliform count in between 30 to 300.

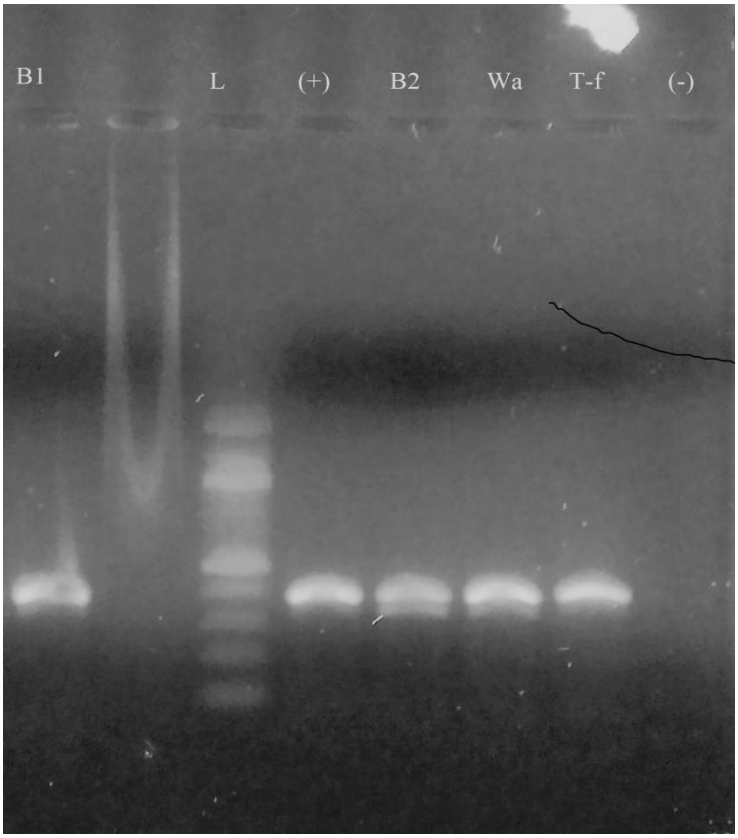


Figure3.8.3: The PCR results. Lanes B represented strains *Salmonella spp*. in Bangshal zone, Wa represented strains *Salmonella spp*. in Wari zone, T-f represented strains *Salmonella spp*. in Tea stall (Filter water). The four positive bands came from the samples that had fecal coliform count in between 1 to 30.

From 110 samples, twelve samples were positive for *Salmonella spp*. Among these twelve samples two samples with positive bands had been appeared from Bangshal zone; one from November and another one from January. Furthermore, two samples with positive bands had been observed from Tea stall (Tejgaon); two from December. Another eight samples with positive bands had been appeared from Jurain, jatrabari, Wari, Khilgaon, Gandaria, Korail Slum, Lalmatia and Narayangonj. The nearness of fecal coliform checks and *Salmonella spp.* shown the fecal pollution and possible survival of the bacterial populace in the water supply line just as

treated water. From the outcome one might say that none of the water tests tried was seen to be consumable dependent on fecal coliform checks and the nearness of pathogenic microorganisms *Salmonella spp*. In spite of the fact that *Salmonella spp.* was not explicitly stable in water conditions and its quality for the most part demonstrated later fecal pollution (WHO 2008). Fecal coliform include in water tests reflected absence of proficiency in water treatment. Where people group water supplies are unchlorinated, they will contain colossal amounts of fecal coliform microscopic organisms, which might be of obliged sterile noteworthiness. Regardless, it may be said that the DWASA water by one way or another gets polluted in the wake of entering the conveyance chain despite the fact that they are dealt with adequately (Mrityunjoy, 2011). As the raised includes showed up in separated water than standard benefit of drinking water one might say that filtration probably won't be totally compelling and the explanation for it may be issue with channel machine or absence of local cleanliness. It appears from the investigation that customers didn't know about the element of contamination in the DWASA faucet water and its impact on general wellbeing, else they would not use this water for drinking, washing utensils and servings of mixed greens. This finding prescribed the need of standard watching the nature of the DWASA water at the client end and to find a way to fix the issue right away.

Chapter 4

Discussion

Presently a day, waterborne sicknesses are one of the genuine general medical issues in Dhaka city. The vast majority of them are endemic and consequently early location of these pathogens can be critical .So, the motivation behind this investigation was to disconnect and recognize the nearness of the *Escherichia coli* and *Salmonella spp.* from crude and locally treated water including bubble and separated water in Jatrabari, Wari, Bangshal and Khilgaon territory of the Dhaka city Typhoid fever and loose bowels are the most unmistakable issue that Dhaka city fights with on a regular premise. City like Dhaka where 20 million of individuals are living required safe water for drinking and for other family unit work. A largeportion of the general population of this city drink water by bubbling or by filtration process. The reason is the crude water that is provided isn't appropriate or safe to drink. So it must be treated by bubbling or filtration. Yet at the same time individuals experience the ill effects of water-borne sicknesses. Several individuals rush to medical clinics and private practices to discover a solution for the runs and other gastrointestinal ailments. Families living beneath the neediness line are at the most elevated danger of these infections as they don't approach safe sustenance and water. Because of constrained measure of cash, even basic treatment for loose bowels can turn into a weight. It is in cases like these where an instance of looseness of the bowels can prompt the passing of a life People group to Dhaka city from everywhere throughout the nation looking for different chances. Thus, the number of inhabitants in the city is expanding always. There are numerous issues that the city manages on an ordinary premise. Having the option to give clean water to its inhabitants is one of them. As expressed before, many individuals in city experience the ill effects of food contamination. This leads us to accept that the water being utilized for nourishment readiness, cooking or utilization may not be of the most astounding quality.

Coliform microscopic organisms are bar molded gram negative microorganisms which could conceivably be motile. Individuals from the coliform bunch incorporates: *Citrobacter, Enterobacter, Hafnia, Klebsiella* and *Escherichia coli*. Fecal coliform are facultative anaerobic, bar molded, gram negative microorganisms. They don't shape spores and can develop within the sight of bile salts or other comparative specialists. Complete coliform microscopic organisms are available in nature. They can be found in soil, vegetation and in the excrement of warm blooded creatures. It very well may be utilized to recognize sullying in water sources. Be that as it may, water sources polluted with vegetation, soil, or influenced surface water would give complete coliform tally. It is all around impossible that a water source that gives complete coliform check is contaminated with sewage. Fecal coliform tally is utilized to identify the presence of sewage in water source. It shows water source that gives a fecal coliform tally is well on the way to contain other pathogenic microscopic organisms.

Access to safe drinking-water is huge as a wellbeing and advancement issue at national, local and neighborhood levels. In specific zones, it has been demonstrated that interests in water supply and sanitation can yield a net financial advantage, as the decreases in antagonistic wellbeing impacts and social insurance costs exceed the expenses of undertaking the intercessions. As indicated by Rules for drinking-water quality by World Wellbeing Association, 1997, any water that is normal for utilization ought not have recognizable fecal coliform, when 100ml of that particular example is tried. Coliform check of the example would choose the nature of the water test.

In this study, PCR was carried out to detect the presence of *Salmonella spp*. in the water samples. This PCR amplifies invA which is a gene in Salmonella. Among From 110 samples, twelve samples were positive for *Salmonella spp*. Among these twelve samples two samples with

positive bands had been appeared from Bangshal zone; one from November and another one from January. Furthermore, two samples with positive bands had been observed from Tea stall(Tejgaon); two from December. Another eight samples with positive bands had been appeared from Jurain, Jatrabari, Wari, Khilgaon, Gandaria, Korail Slum, Lalmatia and Narayangonj. The presence of fecal coliform counts and *Salmonella spp.* indicated the fecal contamination and eventual survival of the bacterial population in the water supply line as well as treated water. From the result it could be said that none of the water samples tested was observed to be potable based on fecal coliform counts and the presence of pathogenic bacteria *Salmonella spp*. From the summary of the results of coliform fecal count in Jatrabari, Khilgaon, Wari and Bangshal also said the same thing as raw water samples which were collected from different houses.The results showed 20% in coliform fecal count (0), 45% in coliform fecal count (1-5), 30% coliform fecal count (6-30) and 5% in coliform fecal count (31-100) from October to the beginning of April. The average fecal coliform count in raw water for Jatrabari zone was 58.33, for Wari 10.17, for Khilgaon 49.83 and for Bangshal 17.86; in boil water 4.25 for Jatrabari, 0.00 for Wari, 4.38 for Khilgaon, for 0.00 Bangshal; in filtered water 1.74 for Jatrabari, 0.00 for Wari, 1.04 for Khilgaon, for 0.00 Bangshal From the bar chart, it clearly seen that Jatrabari zone contained the most fecal coliform counts followed by Khilgaon, Wari and Bangshal for raw, boil and filtered water.

Pipelines run underground to supply water from Dhaka Water Supply and Sewerage Specialist to different family units in various regions and along these lines the reason polluted water may be the issue in the pipeline of this city. The water might be defiled by the spillage on pipeline arrangement of this city. The water might be sheltered at the point it treated however debased when it goes through the pipeline. On the off chance that it occurred, at that point starting

advances ought to be taken by the specialist to change the pipeline arrangement of Dhaka city. From the outcomes it had been obviously seen that the nature of water from the four zones differ a considerable amount. Water from Wari is of the best quality. This is trailed by water from Bangshal and khilgaon. Water from Jatrabari is of the most reduced quality. Jatrabari is a zone that has created a long time prior while Khilgaon and Wari has seen significant improvement over the most recent couple of decades. Pipelines that keep running under Jatrabari may be a lot more seasoned than that of Wari and khilgaon. This implies the pipelines under Jatrabari could be much more harmed than those present in the three different territories. Pipelines of Jatrabari may have significantly more breaks thus a bigger amount of sewage could spill into water supply pipelines.

In this examination the information demonstrates fecal coliform include in treated water. There may be a few explanations behind this. Individuals are uninformed of the term and the temperature at which the water tests were bubbled. Thus, they can't remark on the productivity of the bubbling treatment. Microscopic organisms identified even after treatment may have been thermo tolerant also. Individuals likewise don't have a clue about the sort of channel used to treat water. It may likewise be conceivable that these water medicines are equipped for expelling fecal coliform. In such a case water moves toward becoming debased because of poor taking care of after treatment. Water may progress toward becoming sullied because of poor individual cleanliness. Water may end up tainted from the vessel in which it is put away. Biofilm perhaps present at the base of these vessels may pollute the treated water.

Later on there are more chances to expand this work. This work could be reached out by doing family explicit PCR and species explicit PCR ought to be finished by focusing on a harmful quality. At last, crusades ought to be orchestrated general individuals to prepare them to rehearse

some close to home cleanliness to forestall waterborne maladies. Hands must be kept clean by washing completely with cleanser and water or utilizing a liquor-based hand sanitizer. Cuts and scratches ought to be kept spotless and secured with a wrap until recuperated.

Chapter 5

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