Assessing the Amount of Total Coliform and the Amount of Heavy Metals, Chromium and Lead from Household Water from Different Industrial Zones of Dhaka Division (Tejgaon and Tongi)

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Declaration

It is hereby declared that the thesis project titled “Assessing the Amount of Total Coliform and the Amount of Heavy Metals, Chromium and Lead from Household Water from Different Industrial Zones of Dhaka Division (Tongi and Tejgaon)” submitted by us has been carried out under the supervision of Fahareen Binta Mosharraf, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka. It is further declared that the research work presented here is based on actual and original work carried out by us and it has not been submitted in whole or in part by any other institution for a degree or diploma. Any reference to work done by any other person or institution or any material obtained from other sources have been duly cited and referenced

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Approval

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Abstract

The immensity of the global safe water crisis is underrated, though for decades' water has been contemplated as the most imperative natural finite resources that is required for human development and existence. The accessibility and availability of fresh clean water does not only play a crucial role in economic development and social welfare but also a help to raise awareness regarding potential risk of pathogens and heavy metals from natural water reservoir. *Escherichia coli, Enterobacter, Salmonella* and *Shigella* are one of the major causes of gastroenteritis worldwide. Due to the presence of related genotypic strains, it is reported that natural water bodies can be served as reservoir for the dissemination of these strains in environment. Besides, human are exposed to harmful heavy metals in several ways ranging from consumption of contaminated food and water as well as exposure to polluted air droplets. Nevertheless, innumerable studies have been carried out over the years to assess the impact of disease causing organisms in water quality management, our scientific research showed renewed interest to standardize and evaluate the qualitative as well as quantitative assessment of water quality. In such instance the focus of this experiment is to identify the presence of *Escherichia coli., Enterobacter, Salmonella* and *Shigella***,** along with presence of toxic chemicals like Lead (Pb) and Chromium (Cr). The presence of high concentration of the heavy metals in water may posture substantial health risks along with the impact caused by *Escherichia coli, Enterobacter, Salmonella* and *Shigella.* So constant checking of both chemical and biological quality of natural water sources is highly recommended. The literature reported here focused on a parallel assessment of the levels of the toxic chemicals like Lead and Chromium in distinct locations Tongi and Tejgaon Industrial Zone, Dhaka city within the period of May to December, 2019 along with the presence of *Escherichia coli., Enterobacter, Salmonella* and *Shigella.* Finally, the results portrayed that the in presence of harmful chemicals organisms like *Escherichia coli., Enterobacter, Salmonella* and *Shigella* can sustain for a longer time in water which can show long term impact on human and environment that is yet to be studied at in vivo condition considering the fact how this factors will be associated with human exposure to wastewater in the industrial zone.

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This piece of work I accomplished in pursuance of my B. Sc. dissertation happens to be the first undertaking of this nature I have ever been exposed to. It can be a small step as such but for me it was a great leap. While carrying out my experiments, I faced repeated failures for which I needed help and encouragement not to be frustrated. By good fortune, there were people around me who provided the needed supports.

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Isra Binte Islam **Shamaila Alamgir**

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List of Acronyms

WHO	World Health Organization
WQI	Water Quality Index
DEPZ	Dhaka Export processing zone
IHME	Institution for Health Metrics and Evaluation
DALYs	Disability Adjusted Life Years
E. coli	*Escherichia coli*
IL	Interleukin
TNF	Tumor Necrosis Factor
MIP1	Macrophage Inflammatory Protein
USA	United States of America
Mgm	Milligram
log	Logarithm
Conc.	Concentration
Avg.	Average
ppm	Parts per million
SD	Standard Deviation
STEC	Shiga Toxin Producing *Escherichia coli*
HUS	Hemolytic Uremic Syndrome
ETEC	Enterotoxigenic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
CFU	Colony Forming Unit
TNTC	Too Numerous To Count
EMB	Eosin Methylene Blue

XLD	Xylose Lysine Deoxycholate
MR-VP	Methyl Red – Voges Proskauer
T1N1	Tryptone Salt Agar
TSI	Triple Sugar Iron
MIU	Motility Indole Urea hydrolysation
AAS	Atomic Absorption Spectrophotometer
ATPase	Adenosine Triphosphate
Fur	Ferric uptake regulation protein
MntR	Manganese transcriptional Regulator
PerR	Period protein
ArsR	Arsenical resistance operon Repressor
CueR	Copper Efflux Regulator

Chapter 1

Introduction

The basic need for the existence of different types of living organism is safe drinking water. The three percent of global fresh water is large enough to meet the requirement of humans for millions of years (Srinivas J.1, 2013) [1]. Water is important for the cellular homeostasis and life. It helps to provide nutrient in every living cell and to regulate the internal body temperature by sweating and respiration and flush the waste through urination. Therefore, sufficient amount of safe drinking water is needed to lower the health risk and ensure a better standard for living. As a matter of concern, the accessed sources of water are mostly contaminated due to some natural and manmade causes. The 1/3rd of the population depends on ground water in residential and industrial areas (Srinivas J.1, 2013) [1]. According to research, the drinking water and surface water are getting exposed to industrial effluents and fertilizers that is exfoliating the characteristics of water rapidly (Sunjida SB, 2016) [2]. Developing countries are experiencing poverty that tends to occur through rapid industrialization and urbanization which is ultimately causing water pollution in wide areas. At some point they are concentrating to eliminate poverty risking life of common people knowingly or unknowingly. Developed countries like China has been experiencing environmental degradation, including water pollution since the year of 1970 and they found rapid urbanization and industrialization as the major reason that is causing water contamination (Wang Q., 2016)[3].

The developing countries are spreading awareness everywhere and also taking initiatives to turn over the absurd situation in every possible way. The World Health Organization (WHO) is concerned with public health which is considered as the most important initiative of United Nations. Based on their extensive research, the exact value of household water and drinking

water is identified. There are essentially four different water properties that needed to assess whether or not the liquid is risk-free. It is quite difficult to examine the quality of the water sample based on those four features, so WHO has created a generic test to identify water sample as risk-free. Exterior property of household water includes pH, turbidity, hardness, and others that can be readily monitored and must be tested for household water. The existence of micro-organisms can never be prevented, however different water management methods would be able to maintain the concentration of organisms in water. All of these are included in water biological products, and WHO advises avoiding the existence of dangerous species such as Coliform bacteria, *Bacillus, Vibrio,* Viruses, and Protozoa, respectively. Such bacteria will infect people living in industrial areas if they stay in touch with such contaminated water. Basically, the WHO has already recommended the required amount of pathogens that will not harm the health situation. However, maximum industrial waste contains toxic chemicals such as Mercury, Cadmium, Chromium, Lead, and Iron, and when these are water soluble they cause normal chemical property of water to deteriorate, which gradually leads to series diseases. The WHO has identified an appropriate rate for these substances, most of which are deemed to be less than 1mg /L (WHO, Guidelines for Drinking water quality: fourth edition incorporating the first addendum., 2017).

Governments of developed countries are taking strong initiatives for proper treatment of water as they are financially stable that is eliminating health related problems to a great extent. In contrast, South Asian countries like Sri Lanka, Indonesia, India and Bangladesh are still struggling to take steps to get completely treated water as these processes are too expensive and some may not be even aware of the health risks that would occur if the use of contaminated water is continued in day to day life. A huge population of developing countries are not able to determine which water is risk free and which can cause certain death. Most of them are found using water from the closest water body without even knowing

that these water bodies are already contaminated by industrial residues. However, researchers are working day and night to figure out at what limit these water bodies are getting contaminated and trying to figure out easy way to overcome such situations. One of the major water source in Kuala Lumpur and Selangor is the Selangor River which is possible getting contaminated due to decontamination stations, hospitals and industries (Othman F., 2014)[5]. Researchers in Malaysia have been working on the microbiological contamination and according to Interim National Water Quality Standards, the total coliform and *E.coli* presence are high and harmful for outer body contact (Othman F., 2014)[5]. They have shown comparison of total presence of *E.coli* on the basis of different years and unfortunately the result as not appreciable which proves that they have to work really hard to overcome this situation.

Our neighboring country India is also conducting various projects to figure out maximum problem regarding household water. Being a large state, it is getting more complicated for them to conduct study on all different divisions and hence they are still struggling for risk free household water. In the year of 2013 a study was conducted to evaluate Water Quality Index (WQI) of industrial areas of well water samples in Kakinada, Andhra Pradesh, India. Some of the areas showed unexpected result which is quite harmful for living being. The reason behind such result may be due to sewage waters and heavy industrial activity (Srinivas J.1, 2013) [1]. Afterwards, a researcher completed an experiment on household water in Ahmedabad, Gujrat state, India. The study provides an understanding of the chronology of events that took place since 1960 till 2014 and evaluating the quality of water resources (R. M. , 2016) [7]. More or less it has been observed that water pollution is increasing proportionally with the increase of industries. Eventually, impacts of industrial waste on household water has been noticed in a broad range in Bangladesh which is already widely known for its huge dependency on water body as it is the land of rivers. The capital Dhaka

being the most populated division, unfortunately observes more health risk regarding industrial waste. According to an article, water quality in the aquatic body of Dhaka Export Processing Zone (DEPZ) area was studied on the basis of some physiochemical parameters and heavy metal concentrations. (Akter M., 2014)[8]. Most of the samples exceeded the permissible limits which indicates that the aquatic body around DEPZ is highly polluted for the various types of industrial disposal directly to the water body. This is not the only report that has been conducted in Bangladesh. Day by day researches are approaching towards different projects aiming towards the quality of water all over the country. Recently, researchers in Bangladesh are working on animal model to verify the effect of industrial water and unfortunately the result is not favoring the health. As per an article, the result of heavy metal analysis crossed the limits of WHO guideline and the concentration of manganese in the water sample of Tongi was 0.1249mg/l which is even higher than the maximum permissible limit that portraits the devastating scenario of drinking water quality of that specific area (Sunjida SB, 2016) [2]. This was the analysis of just a single area so this is a matter of concern that what could be the situation of the rest of the country.

Water is contaminated by many sources. It can the microbial presence or the wastes dumped in the main source. Also, water being a very good solvent many heavy metals are dissolves in water which destroy the water quality. Metals are considered as toxins and once they enter the body in more than the prescribed limit, they begin to harm. Untreated or purportedly treated industrial effluents and sewage water contain variable amounts of heavy metals, for example, arsenic, lead, nickel, cadmium, copper, mercury, zinc, and chromium which is broadly studied and their consequences for human wellbeing are routinely evaluated by international bodies such as the WHO. In the result of trace metals, the permissible limit of the content of arsenic, lead and manganese in the surface water samples was found to be <0.05 mg/L whereas the WHO permissible limit of cadmium is 0.005 mg/L [4]

Table 1: Maximum Permissible Concentration of various metals in water for the protection of human health [4].

Metal	Chemical Symbol	Mgm^-3
Lead	Pb	5
Chromium	Cr	50

1.1 Impact of Heavy Metals on Human Health:

Lead: Lead is found in earth's crust and is naturally occurring toxic metal. It is used widespread which has resulted the extensive contamination in the environment. It has been estimated by the Institute for Health Metrics and Evaluation (IHME) in 2017 that lead exposure accounted for 1.06 million deaths and 24.4 million years of healthy life lost (disability-adjusted life years (DALYs)) worldwide due to long-term effects on health and the highest burden was recorded in the under developed countries. In 2016, IHME also estimated that lead exposure accounted for 63.2% of the global burden of idiopathic developmental intellectual disability, 10.3% of the global burden of hypertensive heart disease, 5.6% of the global burden of the heart disease and 6.2% of the global burden of stroke. According to WHO lead is 1 out of 10 chemicals that can cause major public health concern. Prenatal exposures to lead at concentrations lower than recognized a decade ago adversely affect cognitive, neurobehavioral, and neurophysiological development. (Mahaffey)[12]. Lead activates the secretion of the chemokine IL-8 and impacts mitogen-dependent activation by increasing the secretion of the proinflammatory cytokines IL-6 and TNF-α and of the chemokines IL-8 and MIP1-α in the presence of phytohemagglutinin. The recorded changes in gene expression affected major cellular functions, including metallothionein expression, and the expression of cellular metabolic enzymes and protein kinase activity. (Gillis, 2012)[13] Cellular responses to lead correlated with blood lead levels and were significantly

altered in individuals with higher lead content resultantly affecting the nervous system, the negative regulation of transcription and the induction of apoptosis. In addition, change in gene expression in individuals is discerned with elevated zinc protoporphyrin blood levels and found that genes regulating the transmission of nerve impulses were affected in these individuals. (Gillis, 2012)[13]

Chromium: Chromium naturally occurs in ultramafic and serpentine rocks as chromite ($FeCr_2O_4$). It is the 17th most abundant element on earth. It can make complex with other metals like Crocoite ($PbCrO_4$). It remains unstable in presence of oxygen in environment, thus exposing in air it produce an oxide layer that is impermeable for further oxygen contamination. The usage of chromium is vast in industries like plating, alloying, tanning of animal hides, textile dyes, pressure treated lumber, inhibition of water corrosion. For the huge usage the environmental contamination has increased consequently and become a threat in the last years. The effect of chromium in environment is huge. It may enter through the Cr-containing rocks in natural waters which is discharged from leaching of soils or industrials operations. Chromate and dichromate are the forms of Cr (VI) which are extremely soluble under all pH conditions and their recommended limit of the concentration is 1 $\mu g\, L^{-1}$. On the other hand, for industries the range of the concentration is from 2 to 5 $g\, L^{-1}$. In soil, Chromium is deposited in respect of the natural composition of rocks and the composing sediments. Thus the concentration of it in soil varies from place to place. Chromium in soil can increase by dumping soil wastes as chromium bi-products, chromium bearing liquids, chromium plating baths and most importantly by atmospheric deposition. In soil it is present as both Cr (III) and Cr (VI) and goes through a lot of transformations. Cr (VI) is more persistent than Cr (III) and can be sediment for years when there are low level of organic matter present. In plants, Cr (III) provides toxicity in wheat, oat, sorghum plats and Cr (VI) in seedlings.in nutrient solution the Cr is toxic to the agronomic plants at about 0.5 to

5.0 Mgm L−1 and in soil it is toxic to 5 to 100 mg g−1wheras for normal condition the Cr concentration is less than 1 µg g−1. In animals, for certain circumstances Cr (VI) can act like a carcinogen though it is not a classified carcinogen, generally. Furtherly, it can cause allergic reaction, nose bleeding, ulcers, kidney and liver damage and weakens the immune system. Cr (III) can cause skin problems like rash or itching. The developed countries like USA contains chromium in their water supplies less than 5 µg/liter when in general the concentration of chromium in groundwater should be <1 µg/liter. Whereas, In Canadian survey they found 2 µg/liter in median level and in raw water it is 14 µg/liter. In developing countries like Bangladesh, the concentration of chromium in water sample dissolved is 2656–5420 mg/L. this is intensely high when compared with the standard this it 2 mg/L (ECR 1997). Chromium may enter the natural waters by weathering of Cr-containing rocks, direct discharge from industrial operations, leaching of soils, among others. The health hazard associated with exposure to chromium depends on its oxidation state, ranging from the low toxicity of the metal form to the high toxicity of the hexavalent form. Hexavalent chromium [Cr (VI)] is a toxic industrial pollutant that is classified as human carcinogen by several regulatory and non-regulatory agencies. (Tchounwou P B, 2012)[18]

The most commonly and deadly pollutant in the portable water in developing countries are of biological origin. WHO stated that the “Infectious disease caused by pathogenic bacteria, viruses and protozoan or by parasites are the most common and widespread health risk associated with drinking water. One study using 1986 data estimate that 10 major waterborne diseases are responsible for over 28 billion disease episode annually in the developing countries. Of these, diarrheal diseases are the bigger killer. Esrey et al., surveyed 142 studies on 6 of the major waterborne disease and estimated that in developing countries (excluding China), there were 875 million cases of diarrhea and 4.6 million death annually in the mid-1980s. According to the World Bank estimate, more than 3 million children below age 5 die

annually from diarrheal disease contracted through drinking water in the developing world 29. Although the quality of data on mortality and morbidity from unsafe drinking water is mixed (the estimate made by different experts of annual global child vary by almost a factor of 2, from 2 to 4 million), the magnitude of the mortality and morbidity from waterborne diarrheal disease unquestionably make them the planet's biggest environmental health threat to populations. The method to isolate and enumerate the organism is complex, expensive, time consuming, and specific to each organism. In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water system. The principal objectives of municipal water are the production and the distribution of safe water that is fit for human consumption. Coliform organism have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. The term "coliform organisms" refers to Gram-negative, rod shaped bacteria capable of growth in the presence of bile salts or other surface-active agent with similar growth-inhibiting properties and able to ferment lactose at 35-37ºC with the production of acid, gas, and aldehyde within 24-48 hours. They are also oxidase-negative and non-spore-forming and display beta galactosidase activity. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia, Citrobacter*, *Enterobacter,* and *Klebsiella.* However, as defined by modern taxonomical methods, the group is heterogeneous. It include lactose fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii*, which can be found in both feces and the environment(nutrient-rich water, soil, decaying plant material) as well as in drinking water containing relatively high concentration of nutrients. The coliform test can be used as an indicator both of treatment efficiency and of the integrity of the distributing system. Although coliform organism may not always be directly related to the

presence of fecal contamination or pathogens in drinking –water, the coliform test is still useful to monitoring the microbial quality of treated piped water supplies.

1.2 Impact of Microorganisms on Human Health:

***Escherichia coli*:** The *E.coli* bacteria is mostly harmless but some of the strains are able to cause disease. If it is found in the sample of drinking water than it states about the fecal contamination which is of greater risk. Steyn et al reported that the presence of *E. coli* in water also represents useful indicator of risk of infection to users (Rajendra S., 2012). In WHO guidelines *E.coli* is suggested as in index organism in the list of waterborne pathogens. (Gruber. J. S, 2014); In recent studies it is found to be the leading reason of moderate to severe diarrhea in middle income countries. They entre in human body through interaction with food and environmental compartments. *E.coli* present in the drinking represents threat of enteric pathogens and diarrheal disease. To prevent the contamination rate several hygiene process is carried out. Yet, its contamination in hands is found in 2-3 log colony forming unit increased within a few moments. It is found that children less than 5 years are died due to *E.coli* contamination at a rate 0f 5.5% (NAvab-Danaeshmand. T, 2018). In Bangladesh, the tube well waters were tested where it is found in 333 tube wells out of 3186 at a percentage of 10.5%.The point of user of water is more contaminated than the source of them. Out of 6478 samples were tested and it is found in 2179 which is at a rate of 34.7% (Mahmud. Z. H, 2019). *E.coli* O157:H7 is a common strain of *E.coli* that is found creating bloody diarrhea (coliform bacteria and drinking water, 2016). It is the stereotype of shiga toxin producing *E.coli*. It cause severe foodborne disease. Raw milk, meat products and sprouts are few ways to transmit it in human body. Shiga toxin is produced by STEC. They can grow in acidic foods where the pH is 4.4 at an optimum temperature of 370C. In addition, it is found in foods with minimum water activity, aw 0.95 (WHO, 2018). Further, *E.coli* 0111 strain causing hemolytic uremic syndrome (HUS) which is responsible for renal failure and anemia.

The other strains of *E.coli* are; ETEC, cause cramps, nausea, headache and EIEC which cause watery diarrhea.

Enterobacter: *Enterobacter* species are not commonly found as pathogens. Yet now a days they are found to cause community acquired disease and urinary tract infections along with bacteremia. They are found from the feces of animal specially, monkey. *Enterobacter amnigenus* is mostly found in water. The species of *Enterobacter* are found to be the fourth most common gram negative bacteria to cause infection according to the study of the National health Safety Network, USA (J.P.S., 2010). Now a days the motility rate is 20% whereas it can upto50% when an infant is infected with it (WHO, Microbila Fact Sheets). Moreover, *Enterobacter cloacae* are isolated form human clinical specimens. It is famous for causing nosocomial infection for the past few years. It can produce biofilms and cytotoxins which help in their pathogenicity (Izdebski et al., 2014). *Enterobacter aerogenes* is found significantly a paradigm of opportunistic bacteria.

***Shigella*:** *Shigella* is a bacteria genus of Enterobacteriaceae family which is Gram-negative, possible anaerobic, non-spore-forming, non-motile, rod-shaped and biologically closely related to *E. coli. Shigella* is the etiological agent of bacillary dysentery or shigellosis. *Shigella dysenteriae* cause the bacillary dysentery by producing Shiga toxins. *S. flexneri* and *S. sonnei* cause dysentery with having fever, violent abdominal cramps and rectal urgencies. The stool passed found with erythrocytes, polymorphonuclear neutrophils and mucus which is caused due to the invasive character of *Shigella dysenteriae.* It also cause intestinal perforations, septicemia and toxic megacolon. *S. boydii* cause fever that can peak 40^{o}C and an acute colitis. *Shigella* propagates in the gastrointestinal tract at 37^{o}C to reach the colonic epithelial surface. In the *Shigella* infected patient the bile acids are secreted for which the fecal steroid levels are increased in high number along with cholic acid and chenodeoxycholic acid. (Marteyn S. B., 2012). *Shigella* affecting an annual 80–165 million

cases (A, 2016) [20]. It is believed that the number of deaths it triggers every year is between 74,000 and 600,000 (A, 2016) [20]. In African and South Asian, it is one of the top four pathogens that cause moderate to severe diarrhea.

***Salmonella*:** *Salmonella* is one of the most important causes of gastroenteritis worldwide. The symptoms of infection include fever, abdominal pain, diarrhoea, nausea, and sometimes vomiting. Waste water is the main reason of the outbreak of *Salmonella* in the developed countries. (Kovacic A., 2017) The ingested bacteria multiplies in the intestine of host and invades the gastrointestinal tract to cause salmonellosis. In a study is it found that to cause the disease only 103-108 cells of *Salmonella sp.* is enough. (Rahman, 2017)**.** There are now around 2000 serotypes in the genus *Salmonella*, which can affect a wide range of warm- and cold-blooded species. Such diseases can be asymptomatic, although two specific characteristics are known when illness arises in humans. One phenomenon is consistent with severe reticulo-endothelial disease, bacteremia, and persistent pyrexia, that is, 'enteric fever,' and is typical of infections caused by *Salmonella typhi* and *Salmonella paratyphi* A and B. Other serotypes, are *Salmonella sendai, Salmonella cholerae-suis,* and *Salmonella dublin* which also cause septicemia but in addition are often associated with metastatic abscesses. The other, more common, clinical manifestation of enteritis accompanied by fever is caused by a wide variety of serotypes (WHO, Enteric infections due to *Campylobacter, Yersinia, Salmonella,* and *Shigella.)[*21].

Chapter 2

Materials and Methods

2.1 Working Laboratory:

The biological properties of the sample water were administrated by the isolation of *Escherichia coli*, *Enterobacter*, *Shigella* and *Salmonella* in the Microbiology Specialized Research Laboratory, Department of Mathematics and Natural Sciences, BRAC University, Dhaka and the selective Heavy Metal Analysis has been conducted in the Wazed Miah Science Research Centre, Jahangirnagar University.

2.2 Organisms Isolated:

In this study, work was done for the isolation of the 4 specific Coliform organisms under the Enterobacteriaceae group, *Escherichia coli. Enterobacter*, *Shigella* and *Salmonella.*

2.3 Zone of Sample Collection

The water sample was collected randomly from sub-locality of Tongi and Tejgaon Industrial Area, Dhaka, Bangladesh where the dominant source of water used for household purposes is from tube wells and tap installed in the households of these localities. Around 7 different zones were selected from each location respectively and water samples were collected in autoclaved container.

2.4 Weather Forecast

Location	Temperature	Humidity	Precipitation	Wind
Tongi	**30°C**	**79%**	**16%**	**23km/h**
Tejgaon	**29°C**	**74%**	**24%**	**16km/h**

2.5 Methodology

2.5.1 Preparation of Plating Sample on Nutrient agar medium

Within 24 hours of collection of water it was started processing as there is high possibility of deterioration of water quality with the passing hours.

i. Raw water sample was platted into Petri dish containing freshly prepared nutrient agar medium following the spread plate method. The sample was then diluted into dilution factor 10^{-1}, 10^{-2}, and 10^{-3} with normal saline. For each dilution factor different Petri dish containing nutrient agar medium was taken and platted in same way following spread plate method.
ii. The Petri dishes were kept in the incubator for incubation at 37^{o}C for 24 hours.
iii. After incubation, microbial growth was observed on the Petri Dish and CFU/ml was counted from all the plates

2.5.2 Preparation for Plating the Sample in MacConkey Agar Medium

i. The sample diluted in normal saline to the dilution factor 10^{-1} was platted on the MacConkey Agar Medium by spread plate method.
ii. The plates were incubated for 48 hours at 37^{o}C in the incubator and after incubation Plates were observed with identical colonies.

2.5.2.1. Confirmation of *Escherichia coli, Enterobacter, Salmonella* and *Shigella* on MacConkey Agar Medium

Escherichia coli gave a pink colony in MacConkey Agar medium due to precipitating the bile salts in the medium. Similarly, *Enterobacter* utilized the enzymes beta-galactosidase and beta-galactosidase permease and activates the pH indicator for which it showed red stain colonies in MacConkey Agar Medium. *Salmonella* and *Shigella* show colorless and

transparent colonies in MacConkey Agar Medium as they do not ferment lactose present in the medium.

2.5.3. Preparation for Plating Sample in Xylose Lysine Deoxycholate (XLD) Agar Medium

i. The sample was diluted in normal saline to the dilution factor 10^{-1} and platted on the XLD Agar Medium by spread plate method.
ii. After incubation for 48 hours at 37^{o}C in the incubator the Plates were observed with identical colonies.

2.5.3.1. Confirmation of *Escherichia coli, Salmonella and Shigella* in XLD Agar Medium

Escherichia coli fermented the lactose present in medium preventing the pH reversion by decarboxylation and the medium is acidified which showed the *Escherichia coli* colonies as yellow. *Salmonella* can ferment the Xylose sugar and turns yellow but it can decarboxylate lysin which turns the color of colony as pink. In addition, they produce Hydrogen sulfide by metabolizing thiosulfate for which the colonies are found with black centres. *Shigella* can no ferment the Xylose sugar so their colonies remain pink in the XLD Medium.

2.5.4. Preparation for Plating the Sample in Eosin Methylene Blue (EMB) Agar Medium

i. The sample was diluted in normal saline to the dilution factor 10^{-1} and platted on the XLD Agar Medium by spread plate method.
ii. The plates were incubated for 24 hours at 37^{o}C in the incubator and Plates were observed with identical colonies after incubation.

2.5.4.1. Confirmation of *Escherichia coli* and *Enterobacter* on EMB Agar Medium

Escherichia coli colonies give green sheen on EMB Agar Medium for metachromatic properties of dye and its ability to ferment and creating acid end products. *Enterobacter* gave mucoid purple colonies for fermenting lactose present in the medium. Sometimes the colonies were also found nucleated.

2.5.5 Biochemical Identification

Afterwards, for further confirmation Biochemical tests were run which is a time consuming process. For biochemical tests, fresh batch of sample organisms was chosen which was sub-cultured from the previous batch.

i. Indole

- Bacterial colony was inoculated in indole broth from Nutrient Agar with the help of sterile loop and kept in incubation for 48 hours at 37^{o}C.
- After incubation period Kovac's Reagent was added five drops and few minutes were waited.
- This test is carried to observe whether the bacteria can convert tryptophan into indole by using the tryptophanase enzyme. The result is positive when red color layer is observed at the surface of the broth and a negative result shows yellow appearance.

ii. Methyl Red

- A single bacterial colony taken from Nutrient Agar Medium and inoculated at MR-VP broth with a sterile loop and incubated for 24 hours at 37^{o}C in incubator.
- Methyl Red Reagent is added 2-3 drops and instant color change is observed.
- The bacteria if can ferment the acids present with glucose will give a red color at the surface of the broth, which indicates it to be methyl red positive. On the

other hand if it cannot ferments the acids than it will give a yellow color appearance in the surface, taking the bacteria to be methyl red negative.

iii. Voges Proskauer

- In MR-VP broth a Bacterial colony was added with sterile loop and incubated at 37^{o}C for 48 hours in shaker incubator. The reagents Barrits A and Barrits B are added five drops each and waited for 5-10 minutes to see the result.
- This test is performed to observe the ability to produce acetoin, the main end product of glucose metabolism pathway. If the tested organism is positive it will give a red color (alpha napthol acts as catalyst) on the surface of the broth and for negative result there will be a yellow shade on the surface of broth.

iv. Citrate Utilization

- Bacterial colonies were picked up from Nutrient agar plate by a straight wire and inoculated into the slope of Simmon's citrate agar (Difco, USA) and incubated at 37^{o}C for 24 hours.
- If the organism had the ability to utilize citrate, the medium changed its color from green to Prussian blue and for a negative result the slant would have no growth of bacteria and would remain green.

v. Oxidase

- This test is performed to observe if the bacteria can utilize the enzyme oxidase.
- From a fresh nutrient agar plate bacteria is taken with a sterile loop on a piece of filter paper.
- The oxidase reagent is added 1-2 drops over the bacteria on filter paper.

- For positive reaction the bacteria turns violet within few seconds and for a negative result no change in color observed.

vi. Catalase

- Hydrogen peroxide breaks down into oxygen and water in presence of catalase enzyme.
- A loopful bacteria taken in glass slide with loop from nutrient plate.
- Bubbles formed for the presence of hydrogen in a positive result and for negative result no change observed.

vii. Motility Indole Urease Test

- The motility of the organism is observed through this test along with the ability to break down urea in the presence of urease and indole production.
- It is a semi solid media and a bacterial colony is taken from the nutrient plate with a sterile needle and inoculated in it.
- The positive result for motility is observed by the diffused growth around the stabbed line. Growth within the stabbed line is taken negative result for motility.
- Urease presence turns the whole media into pink from orange. And a negative result shows no change in color of the media.
- For presence of indole a cherry-red ring is observed on the surface of the media. A yellow or brown color is observed as the negative result.

viii. Triple Sugar Iron

- Triple sugar iron test is perform to observe if the bacteria can utilize glucose, lactose, sucrose and produce gas and hydrogen sulfide.

- With a sterile needle bacterial colony is connected from fresh grown nutrient agar plates and inoculated in the TSI medium and incubates for 24 hours at 37^0C.
- The organism fermenting only glucose and not lactose or sucrose will show the color change in slant but no color change in butt. The organism fermenting all the three sugar shows the change of color in butt and slant from red to yellow for the change in alkalinity to acidity.
- The presence of hydrogen gas creates bubble of crack in the media. If no such found than no gas is produced.
- The butt of the media turns into deep black for the presence of hydrogen sulfide. A negative H_2S production results no such thing.

ix. Dextrose Fermentation

- Phenol red dextrose broth is prepared with the pH indicator phenol red. Bacterial colony is taken using sterile loop from nutrient plate freshly prepared and inoculated in the broth.
- After incubation of 24 hours at 37^0C the broth is observed with a yellow color indicating that the organism can produce acid to utilize dextrose. The negative result indicates no color change

x. Lactose Fermentation

- Phenol red lactose broth is prepared to observe the bacteria can ferment the carbohydrate, lactose.
- From fresh nutrient plate, bacterial colony is taken with sterile loop and inoculated in the broth and kept it in incubation at 37^0C for 24 hours.
- Lactose fermenting bacteria turns the media into yellow. The media remaining red color is taken for a negative result.

xi. Sucrose Fermentation

- Sucrose fermentation is observed by inoculation bacteria from fresh nutrient media into the phenol sucrose broth and incubated at 37^0C for 24 hours.
- Sucrose is taken to be fermented when the media turns yellow from red. No color change in broth is taken a negative result.

xii. Gram Staining

- The technique invented by Christian Gram to differentiate between Gram positive and Gram negative bacteria on the presence of peptidoglycan layer in their cell wall.
- From fresh nutrient agar plate, with a sterile loop a bacterial colony is taken in microscopic glass slide and a smear is prepared with heat fixation by using normal slide.
- Crystal violet, the primary stain added on the smear and kept for 45 seconds. Gram's Iodine, the mordant is added after Washed with water for a minute.
- The decolorizing agent, 90% ethanol is used to wash the satin than safranin, the Counter stain added for 45 seconds, washed and air dried.
- The slides observed under microscope, Gram positive bacteria show bluish purple color and Gram negative bacteria show pink color.

2.5.6 Preparation of Stock Sample

T_1N_1 (A-I) agar was prepared for bacterial stock, 2ml in each vial. From a fresh incubated Nutrient agar plate, bacterial colony was taken with sterile needle and stabbed on the age for three times. Then the vial was incubated at 37ºC (Eyela; Tokyo, Japan) for 24 hours. After the incubation, the surface of the medium was covered with 300µml sterile paraffin oil to

stop the growth. The cap of the vial was wrapped with parafilm and stored at room temperature.

2.6 Heavy Metals Examined in the Water Sample

Lead (Pb) and Chromium (Cr)

2.6.1 Heavy Metals Analysis by Atomic Absorption Spectrometer

For heavy metal analysis, atomic absorption spectrum of a sample is applied to determine the presence and concentration of analytes present in the selected sample which was conducted in the Wazed Miah Science Research Center of Jahangirnagar University by using an atomic absorption spectrophotometer (AAS) (Shimadzo, AA7000, Japan). This is mostly accepted as the standard technique for metals determination since it provides accurate result at a low cost (Es'haghi et al., 2011). Hollow cathode lamp for mono element was employed for the determination of each heavy metal. The analysis for the trace metals like chromium (Cr) and Lead (Pb) was done by Shimadzo, AA7000 Flame Atomic Absorption Spectrophotometer. It includes atomizing the analytes in the sample so that they can be analyzed.

Figure 2.6.1(a): AA7000 Flame Atomic Absorption Spectrophotometer

By running different concentrations of standard solutions of all kinds of metals separate curves were formed. A blank sample was analyzed whose result was subtracted from the samples to correct for reagent impurities and errors. For every sample, counts were taken four times and its average values were taken to determine the concentration. A standard curve was prepared by plotting the absorbance reading on Y-axis versus the concentration of each standard solution of metal on X-axis. Then, the concentration of metal was calculated in the sediment samples of interest by plotting the AAS reading on the standard curve.

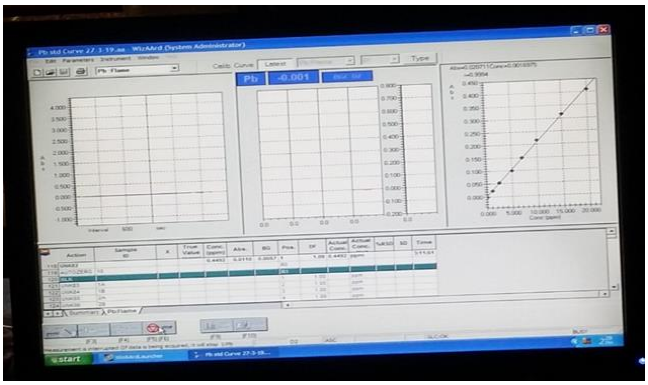

Figure 2.6.1(b): Concentration of Standard Deviation curve

2.6.2 Acid Digestion Procedure of Heavy Metals before running the Sample through AAS

100ml of water sample was acid digested with 10ml of Nitric acid and 5ml of Hydrochloric acid and heated at 200℃ until the volume decreases to 70ml. Then, deionized waster was added to bring back the volume to 100ml. A blank sample was prepared with deionized water and adding same amount of nitric acid and hydrochloric acid as the sample and heated.

Figure 2.6.2: Acid digested samples

2.6.3 Application of AAS:

At first the machine was prepared allowing air-acetylene to pass through the compressed aperture. It is the preferred flame for the determination of approximately 35 elements by atomic absorption. The temperature of the air-acetylene flame is about 2300°C. An air-acetylene flame can be used with all Perkin-Elmer burner heads. (Perkin, 1996) Then all the samples were placed in respective places. A thread like tube was used to suck up sample for about 3ml for each turn. Then the Ignite and Spark buttons were pressed simultaneously to boost up the heat and analyze metals gradually.

Figure 2.6.3: Processing of Heavy metal

Chapter 3

Result

3.1 Samples Collected from Different Points around Tejgaon and Tongi Industrial Area (May-November, 2019)

3.1.1 CFU count on the Nutrient agar plates based on different dilution factor

Zone	Dilution Factor	Sample	Sample	Sample	Sample	Sample	Sample
Tejgaon	**CFU count 10^{-1}**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**
	CFU count 10^{-2}	**1.24x10^5**	**1.12x10^5**	**1.56x10^5**	**1.32x10^5**	**1.09x10^5**	**1.66x10^5**
	CFU count 10^{-3}	**7.8x10^5**	**5.6x10^5**	**6.4x10^5**	**6.1x10^5**	**3.9x10^5**	**7.1x10^5**

3.1.2 CFU count on Nutrient Agar plates based on different dilution factor

Zone	Dilution Factor	Sample	Sample	Sample	Sample	Sample	Sample	Sample
Tongi	**CFU Count 10^{-1}**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**
	CFU Count 10^{-2}	**1.23 X 10^{-5}**	**1.75 X 10^{-5}**	**1.96 X 10^{-5}**	**7.8X 10^{-4}**	**1.68 X 10^{-5}**	**1.07 X 10^{-5}**	**1.34 X 10^{-5}**
	CFU Count 10^{-3}	**3.4X 10^{-4}**	**2.7X 10^{-4}**	**2.4X 10^{-4}**	**3.0X 10^{-4}**	**3.1X 10^{-4}**	**8.0X 10^{-4}**	**3.3X 10^{-4}**

3.1.3 Biochemical test Results of Different colonies for Tejgaon area:

Specimen	Gram	Indole	MR	VP	Citrate	TSI	Motility	Urease	Oxidase	Catalase	Glucose	Lactose	Sucrose	Remarks
S1 c	-	-	-	-	-	S=Y B=Y G=N H=N	+	-	-	+	F= + G= -	F= - G= -	F= - G= -	*E.coli*
S1 d	-	-	+	-	-	S=R B=R G=N H=N	-	-	-	+	F= + G= -	F= - G= -	F= - G= -	*Shigella*
S1 f	-	-	-	-	-	S=R B=R G=N H=P	+	-	-	+	F= + G= -	F= - G= -	F= - G= -	*Salmone*
S1 h	-	-	+	-	-	S=R B=R G=N H=N	+	-	-	+	F= - G= -	F= - G= -	F= - G= -	*Salmone*
S2 a	-	-	-	-	-	S= B= G= H	+	-	-	+	F= + G= +	F= - G= -	F= - G= -	*Shigella*
S3 a	-	-	-	+	-	S=Y B=Y G=N H=N	+	+	-	+	F= + G= +	F= + G= +	F= + G= +	*E.coli*
S3 b	-	-	-	+	-	S=Y B=Y G=P H=N	+	+	-	+	F= + G= +	F= + G= +	F= + G= +	*E.coli*
S3 c	-	-	-	-	+	S=Y B=Y G=P H=N	+	+	-	+	F= + G= +	F= + G= +	F= + G= +	*Enterobc*
S3 d	-	-	+	+	-	S=Y B=Y G=P H=N	+	+	-	+	F= + G= +	F= + G= +	F= + G= +	*Enterobc*
S3 e	-	-	-	-	-	S=Y B=Y	+	-	-	+	F= +	F= +	F= +	*Enterobc*

						G=P H=N					G= +	G= -	G= -	
S3 g	-	+	-	+	+	S=Y B=Y G=P H=N	+	+	-	+	F= - G= -	F= + G= +	F= + G=+	*E.coli*
S4 b	-	+	-	-	-	S=R B=Y G=N H=N	+	+	-	+	F= + G= -	F= - G= -	F= + G= +	*Shigella*
S5 d	-	-	-	+	+	S=Y B=Y G=P H=N	-	-	-	+	F= + G= -	F= - G= -	F= - G= -	*Enterobo*
S5 e	-	-	-	-	-	S=Y B=Y G=N H=N	-	-	-	+	F= + G= -	F= - G= -	F= - G= -	*Shigella*
S6 a	-	-	-	-	-	S=Y B=Y G=N H=N	-	-	-	+	F= + G= -	F= - G= -	F= - G= -	*Shigella*
S6 b	-	-	-	+	+	S=Y B=Y G=P H=N	+	+	-	+	F= + G= +	F= + G= +	F= + G= +	*Enterobo*
S6 c	-	-	-	+	-	S=Y B=Y G=N H=N	+	-	-	+	F= + G= -		F= - G= -	*Enterobo*
S6 f	-	-	-	-	-	S=Y B=Y G=P H=N	+	-	-	+	F= + G= +	F= + G= +	F= + G= -	*Shigella*

3.1.4 Biochemical test Results of Different Colonies for Tongi Area

Specimen	Gram	Indole	MR VP	Citrate	Oxidase	Catalase	Urease	Motility	TSI	Dextrose	Lactose	Sucrose	Remarks
C1	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
D4	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= +	F=+	F= -	F=+	*Salmonella*
H3	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= +	F=+	F= -	F=+	*Salmonella*
H4	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
I2	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= +	F=+	F= -	F=+	*Salmonella*
J1	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
J2	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
J4	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
K4	-	-	-	+	+	-	+	-	+	S= Y B= Y	F=+	F=+	F=+	*Enterobacter*

										G= + H= -				
L1	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
L3	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= +	F=+	F= -	F=+	*Salmonella*
M 2	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= +	F=+	F= -	F=+	*Salmonella*
M 4	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
N1	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
N2	-	-	-	+	+	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Enterobacter*
P2	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
P3	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
Q4	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
S1	-	-	+	-	-	-	+	-	-	S= R B= Y	F=+	F= -	F= -	*Shigella*

										G= - H= -				
T2	-	-	+	-	+	-	+	-	+	S= R B= Y G= + H= -	F=+	F= -	F=+	*Salmonella*
T3	-	+	+	-	-	-	+	-	+	S= Y B= Y G= + H= -	F=+	F=+	F=+	*Escherichia coli*
T4	-	-	+	-	-	-	+	-	-	S= R B= Y G= - H= -	F=+	F= -	F= -	*Shigella*

3.2 Strain Confirmation

Based on the data of biochemical tests mentioned in the book "Microbiology, An Introduction" written by Tortora we state that our desired organism *E. coli. Enterobacter, Salmonella* and *Shigella* are present in different water sample (Tortora G., 2014)[22].

3.2.1 Test results for Coliforms (Tortora G., 2014)

Tests	***Shigella***	***Salmonella***	***Escherichia coli***	***Enterobacter***
Indole	Positive/Negative	Negative	Positive	Negative
Methyl Red	Positive	Positive	Positive	Negative
VP	Negative	Negative	Negative	Positive
Citrate	Negative	Positive	Negative	Positive
Oxidase	Negative	Negative	Negative	Negative
Catalase	Positive	Positive	Positive	Positive
Urease	Negative	Negative	Negative	Negative
Motility	Negative	Positive	Positive	Positive
TSI	Slant: Red Butt: Yellow Gas formation: Negative H2S: Negative	Slant: Red Butt: Yellow Gas formation: Positive H2S: Positive	Slant: Yellow Butt: Yellow Gas formation: Positive H2S: Negative	Slant: Yellow Butt: Yellow Gas formation: Positive H2S: Negative
Dextrose Fermentation	Positive	Positive	Positive	Positive
Lactose Fermentation	Negative	Negative	Positive	Negative
Sucrose Fermentation	Negative	Negative	Positive	Positive
Gram staining	Gram-negative, Rod shaped	Gram-negative, Rod shaped	Gram-negative, Rod shaped	Gram-negative, Rod shaped

After observing the above mentioned tables and from all different samples, presence of *E. coli*. *Enterobacter, Salmonella sp.* and *Shigella sp.* has been confirmed based on biochemical test result.

3.3: Pictures of Biochemical Test Results

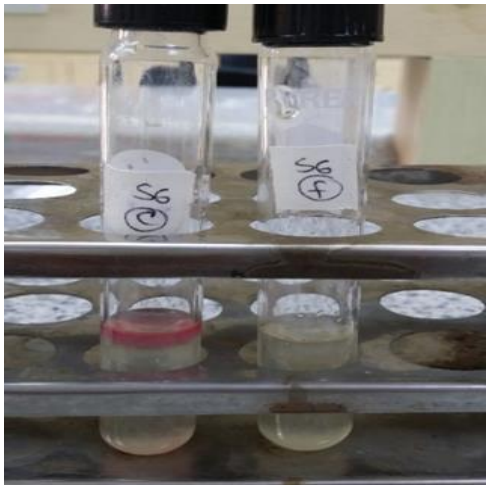

Figure 3.3.1: The sample S6© at the left indicates positive result on the other hand the sample S6 (f) on the right shows the negative result for **Indole test.**

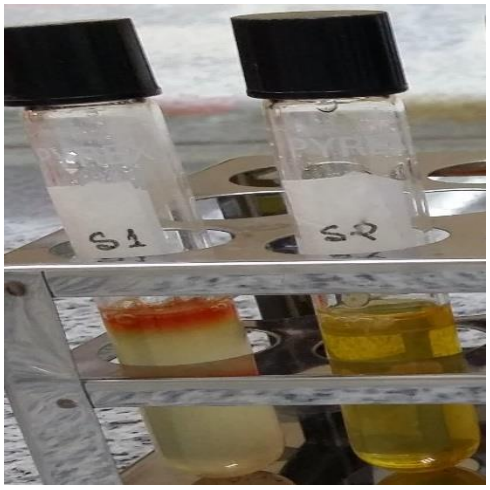

Figure 3.3.2: The sample S1 at left shows positive result on the contrary sample S2 shows negative result for **Methyl Red test.**

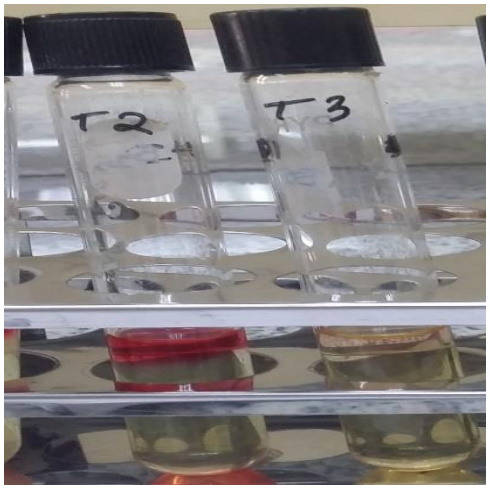

Figure 3.3.3: The sample T2 at the left showing the positive result and the sample T3 on the right shows the negative result for **Voges-Proskauer test.**

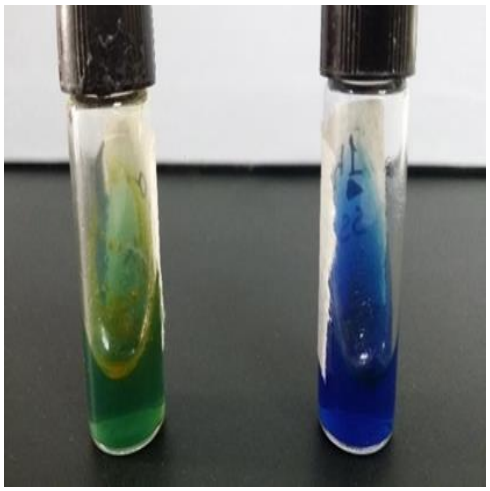

Figure 3.3.4: The sample at the right (blue) showing the positive result on the other hand the sample on the left (green) shows the negative result for **Citrate test.**

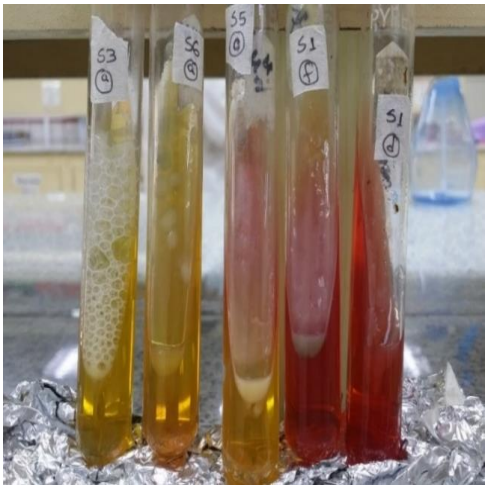

Figure 3.3.5: The sample S3 (a) shows positive fermentation and gas production is also positive, the sample S6 (a) shows only positive fermentation, sample S5 (c) shows partial fermentation, sample S1 (f) and S1 (d) shows negative fermentation result for **TSI test.**

Figure 3.3.6: The sample S4 (b) and S4 (d) shows positive result bur sample S4 (d) shows false negative result for **Catalase test.**

Figure 3.3.7: Here sample J1 shows positive result by turning violet color to the colony and other samples; J2, J3, J4 shows positive results (no violet zone) for **Oxidase test.**

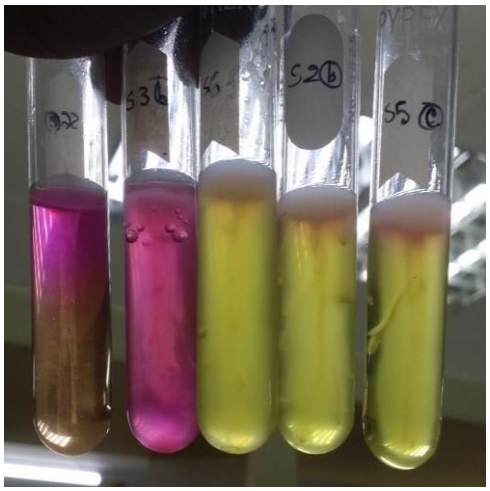

Figure 3.3.8: The 2 samples from left turned pink indicating urease and Motility positive, on the contrary the last 3 samples at the right shows motility but no pink color so they are Motile but Urease negative for **MIU tests.**

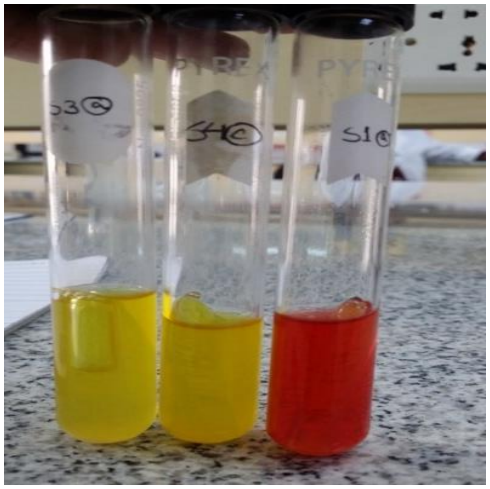

Figure 3.3.9: The sample S3 (a) shows positive fermentation along with gas production. The sample S4 (c) shows positive fermentation but no gas production. The sample S1 (a) is still red so it shows negative result for **Fermentation tests.**

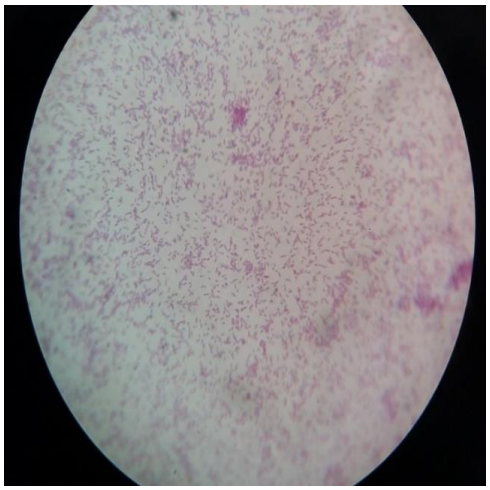

Figure 3.3.10: The sample shows rod shaped and it is showing pink colonies which refers to **negative Gram staining.**

3.4 Result of Heavy Metal Analysis using Atomic Absorption Spectrometer:

3.4.1 Lead

Zone	Sample		Conc.(ppm)	SD	WHO Limit
Tejgaon	Tejgaon 1	**a**	0.00 (-0.080)		0.01
		b	0.00 (-0.106)		
		c	0.00 (-0.116)		
		d	0.00 (-0.100)		
		Avg.	0.00 **(-0.106)**	**0.0151**	
	Tejgaon 2	**a**	0.00 (-0.106)		0.01
		b	0.00 (-0.111)		
		c	0.00 (-0.070)		
		d	0.00 (-0.085)		
		Avg.	0.00 **(-0.100)**	**0.0190**	
Tongi	Tongi 1	**a**	0.00 (-0.131)		0.01
		b	0.00 (-0.126)		
		c	0.00 (-0.126)		
		Avg.	**0.00 (-0.126)**	**0.0028**	
	Tongi 2	**a**	0.00 (-0.059)		0.01
		b	0.00 (-0.044)		
		c	0.00 (-0.008)		
		d	0.00 (-0.0071)		
		Avg.	**0.00 (-0.013)**	**0.0261**	

3.4.2 Chromium

Zone	Sample		Concentration (ppm)	SD	WHO Limit
Tejgaon	**Tejgaon 1**	**a**	0.0175		0.05
		b	0.0166		
		c	0.0156		
		d	0.0213		
		Avg.	**0.0185**	**0.0151**	
	Tejgaon 2	**a**	0.0156		0.05
		b	0.0166		
		c	0.0185		
		d	0.0166		
		Avg.	**0.0166**	**0.0012**	
Tongi	**Tongi 1**	**a**	0.00 (-0.001)		0.05
		b	0.0004		
		c	0.0042		
		d	0.0023		
		Avg.	**0.0004**	**0.0022**	
	Tongi 2	**a**	0.0166		0.05
		b	0.0156		
		c	0.0137		
		d	0.0137		
		Avg.	**0.0147**	**0.0014**	

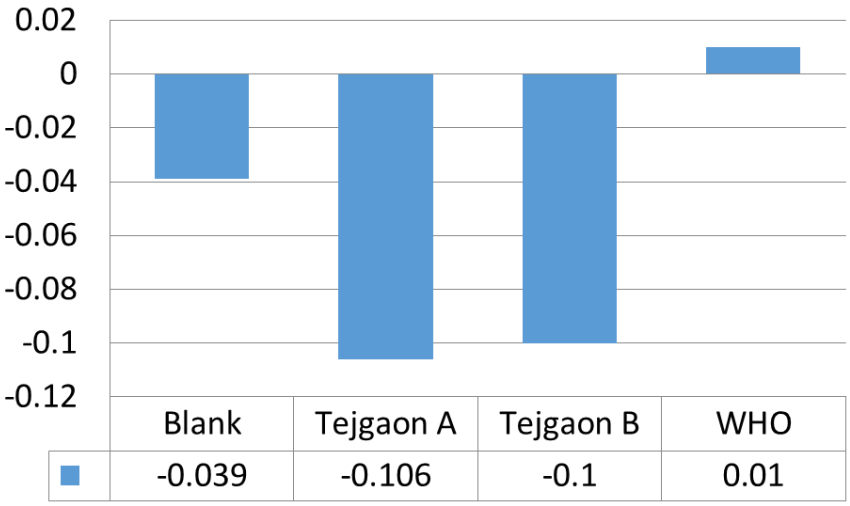

Figure 3.4.1a: Concentration of Lead in Tejgaon

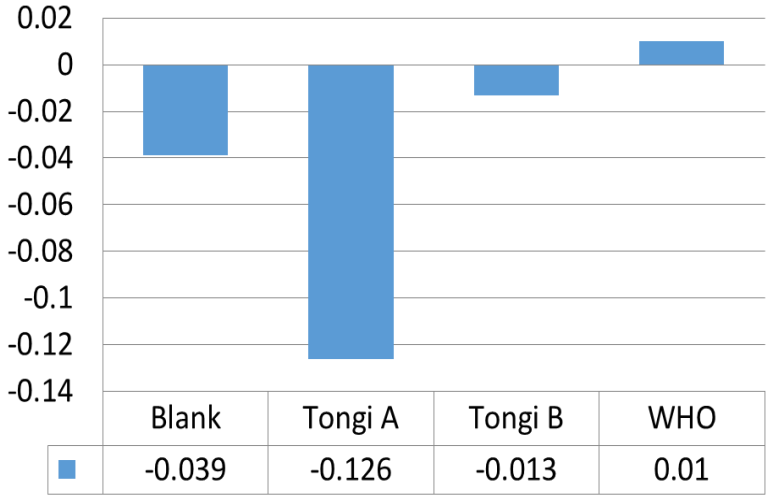

Figure 3.4.1b: Concentration of Lead in Tongi

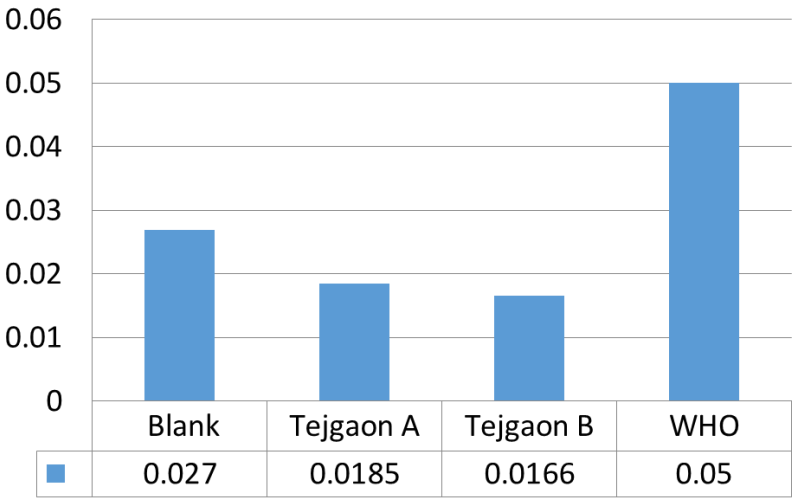

Figure 3.4.2a: Concentration of Chromium of Tejgaon

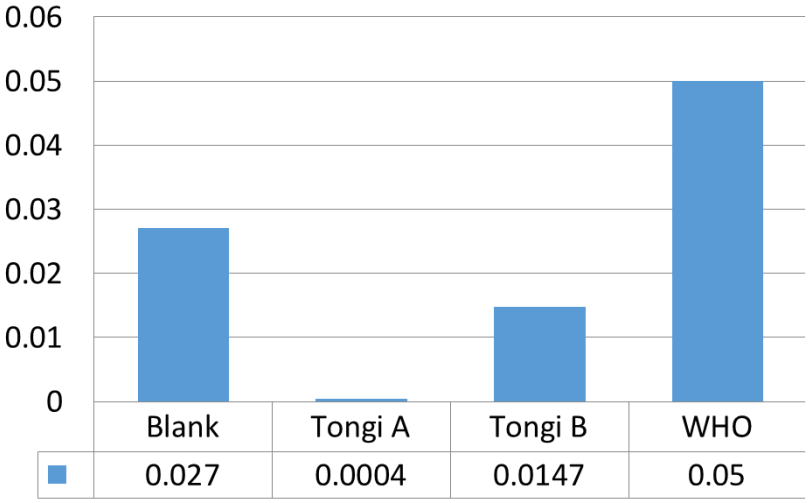

Figure 3.4.2b: Concentration of Chromium in Tongi

Chapter 4

Discussion

Coliforms have become common organism found in industrial water area that is alarming for both human and environment. After demonstrating some essential biochemical tests, it was possible to come to a conclusion where the presence of *E. coli, Enterobacter, Shigella* and *Salmonella* was quite evident. Both of these organism are well known for their virulent capability that causes serious harm on human health. *Shigella,* the human shigellosis causative factor, causes disease in humans, but not in other species. It usually causes dysentery during infection. Moreover, *Salmonella* is no less than other organisms in causing infection in human. Severe reticulo-endothelial disease, bacteremia, and persistent pyrexia, i.e.' enteric fever,' are typical infections caused by *Salmonella typhi* and *Salmonella paratyphi* (WHO, Enteric infections due to C*ampylobacter, Yersinia, Salmonella,* and *Shigella*.)[21]. *E.coli* O157:H7 is a common strain of *E. coli* that is found creating bloody diarrhea which is very common in Bangladesh and the other strain of *Escherichia coli* are; ETEC, cause cramps, nausea, headache and EIEC which cause watery diarrhea whereas *E.coli* 0111 strain causes hemolytic uremic syndrome. Moreover, *Enterobacter* is no less in the competition with other organisms and they are causing great harm worldwide. Based on their severe action we were concerned about their presence in household water of industrial area. *Shigella* was found more or less in every sample which is pointing towards the presence of this organism in abundant in Tejgaon area. However, *Salmonella* was found at the first location only so based on the result it can be said that *Salmonella* may not be frequently found in household water of Tejgaon area.

Though samples were taken from limited areas but surprisingly the amount of organisms found after inoculating in Nutrient agar and Specific Media from both raw and enriched

condition are not acceptable to live a healthy life. Comparatively the amount of *E. coli* and *Enterobacter* isolated from both Tongi and Tejgaon are more than any other organism. By observing the result it is found that the number of *Salmonella* is higher in Tongi than that of Tejgaon which means people of Tongi are getting encountered by *Salmonella* often.

One of the aim of taking water sample from industrial area was to witness weather these organisms can survive in the presence of heavy metal. Differences in the bacterial resistance to different heavy metal are mainly seen due to the different concentration of different heavy metals. For instance, *Shigella* has the mechanism of resistance to lead might be due to an efflux by P-type ATPase and intracellular compounds complexation (D., 1999)[24]. In fact, it has been found that many of the genes affected by metal stress are controlled by metallo-regulatory proteins known as Fur, MntR, PerR, ArsR and CueR (Moore, 2005)[25]. Several bacterial species utilize intra- and extracellular binding mechanisms to avoid toxicity to Pb^{2+}. According to an experiment it was observed that *Shigella* can resist Lead at a lower concentration of 0.5mM but at higher concentration of Lead it cannot resist properly (Onuoha S., 2016)[23]. As per the ASS result, *Shigella* could easily survive at a concentration of -0.106 and 0.100 ppm of Lead respectively that means this concentration did not cause any problem in the growth of *Shigella.* Moreover, in some other experiment *Shigella* sp. were able to withstand the initial dose of 100ppm of Chromium and a minimum amount of absorption of Chromium which is 48.57mg/L was visible that means *Shigella* uptakes Chromium to maintain some metabolic activities. (A. S. S., 2010)[27].

In addition, *Salmonella typhimurium* also shows some unusual but interesting activities in presence of Chromium. Since *Salmonella typhimurium* was unable to utilize several sugars as carbon sources in solid media, they are able to grow in liquid media. Analyzing the agar it was found that Cr^{3+} was present at concentration of 75 µM. The addition of Cr^{3+} to liquid media in 0.5 mm concentrations did not inhibit the wild type or the mutants, but

concentrations as low as 0.01 to 0.05 mm inhibited the deletion mutants which indicates an increased permeability to Cr^{3+}, resulting in higher effective intracellular concentrations (FLORA., 1977)[26]. After observing the result it can be said that concentration of Chromium is vital for the growth *Salmonella*.

E. coli has a negative relationship with Chromium which means it shows inhibitory activities in presence of this metal. In an experiment the growth of *E. coli* was seen decreasing from 77% to 38% due to the shifting of concentration of Chromium from 0.1mM\L to 0.5mM\L which clearly state that Chromium has reverse impact on *E. coli*. (Kalantari N., 2008). Hexavalent chromium is hundred fold more harmful than trivalent chromium and it is recognized as the most dangerous environmental pollutant as it can cause mutations, skin disorders and also lung cancer in humans. When Cr (VI) is converted into Cr (III) then it can be proven as useful process for remediation of Cr (VI) pollution. Transformation of Cr by chromium-resistant bacteria is an eco- friendly option for detoxification of chromium where the mechanism is to convert Cr (VI) into Cr (III) properly. (Shodhganga, 2014). Though Lead is one of the most toxic heavy metal but *E. coli* shows a better amount of resistance against Lead which means the result of the concentration lead found will hardly shows any impact on the growth of *E. coli*. (R. K. S., 2012)

Lastly, *Enterobacter* is not commonly known for its pathogenic activity but recently they are found in water causing urinary tract infections along with bacteremia after intake of contaminated water. Now the matter of concern for us is *Enterobacter* is found also in industrial areas where lots of inhabitants has access every day for living purpose so we need to overview on its relation with heavy metals. The potential of *Enterobacter* to immobilize Lead by enhancing solubilization of insoluble Pb compounds was tested in a research. Significantly an increased Phosphate solubilization is observed by 88.6% in the case of *Enterobacter sp.* thereby enhancing the immobilization of Lead by 14.7–26.4% that proves

that *Enterobacter* is able to reduce Lead toxicity from the environment. That is why at the minimum level of Lead no huge impact was observed in case of *Enterobacter*. (Park H. J., 2010). Finally, while considering about other metals if the relationship between *Enterobacter* and Chromium is considered then it becomes quite obvious that *Enterobacter* decreases the value of Chromium in water quite effectively. The process of heavy metal removal by biological materials is known as bio-sorption and the biological materials used are called as bio-sorbents. Various bio-sorbents like bacteria, fungi, yeasts and agricultural products have been used for biosorption. *Enterobacter cloacae* have been shown to remove chromium from industrial effluent. (Thatheyus J. A., 2016). According to the result it can be estimated that in presence of Chromium no obstacles were observed in the growth of *Enterobacter* rather the concentration of Chromium was not even close to WHO permissible limit which concludes that *Enterobacter* might have tried to remove Chromium from water samples.

Chapter 5

Conclusion

Throughout past years there has been an enormous acceleration of awareness among people concerning water quality all around the world for which new projects are being implemented internationally and satisfactory results achieved. Yet people of the developing countries are still at alarming condition which can turn into more dangerous situation in future. The samples collected from different zones like Tejgaon and Tongi are not free from industrial waste materials. The research work has been studied extensively in terms of relevant biological parameters and the concentrations of some heavy metals. Biological parameters are not within the range of WHO and show an up growing trend which is highly alarming. In most of the samples the values of coliforms organisms exceeded the standard range, which is just out of control. In case of heavy metals, the concentrations of Chromium and Lead were below the permissible limits. A significant correlation also determined about the common source of the metal and also the contamination level of the sampling region. Enrichment factor also shows actual scenario of the water body. According to this study it can be concluded that the household water around industries is highly polluted for the various types of industrial disposal directly to the water body which can urge a huge amount of impact on human. The experiment is regulated in the *in vitro* condition so to know more of the impacts of pollutants we need to do some experiments in the *in vivo* condition which we are hoping to do for further research. The reason to aim further research is because the amount of health hazards the above mentioned coliforms are capable to do is not seen in that amount among the inhabitants of those areas which means there might be chances of mutation either in our desired organism or those people who are in contact of these household water regularly.

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Appendix

Appendix I

Media Composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned, all the media were autoclaved at 121^{o}C for 15 min.

1. **Nutrient Agar**

Ingredients	**Amount (g/L)**
Peptone	5.0
Beef Extract	3.0
Sodium chloride	5.0
Agar	15.0
Distill water	1 Litre

2. **MacConkey Agar**

Ingredients	**Amount (g/L)**
Peptone	17.0
Poly peptone	3.0
Sodium chloride	5.0
Lactose	10.0
Bile Salts	1.5
Agar	13.5
Final pH	7.1±0.2

Neutral Red	0.03
Crystal Violet	0.001
Distill water	1 Litre

3. XLD Agar

Ingredients	**Amount (g/L)**
Lactose	7.5
Sucrose	7.5
Sodium chloride	5.0
Sodium Thiosulfate	6.8
L-Lysine	5.0
Agar	15.0
Xylose	3.75
Yeast Extract	3.0
Sodium Deoxycholate	2.5
Ferric Ammonium Citrate	0.8
Phenol Red	0.08
Distill Water	1 Litre

4. EMB Agar

Ingredients	**Amount (g/L)**
Peptone	10.0
Lactose	10.0
Di-potassium hydrogen phosphate	2.0

Eosin Y	0.4
Methylene blue	0.06
Agar	15.0
Distill water	1 Litre

5. TSB Broth

Ingredients	**Amount (g/L)**
Casein peptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Dextrose	2.5
Agar	15.0
Distill water	1 Litre

6. Indole Broth

Ingredients	**Amount (g/L)**
Tryptone	10.0
Sodium chloride	5.0
pH	7.5
Distill water	1 Litre

7. MR-VP Broth

Ingredients	Amount (g/L)
Peptone	7 g
Dextrose	5 g
Di-potassium hydrogen phosphate	5 g
Distill water	1 Litre
Final pH	6.9

8. Simmon's Citrate Agar

Ingredients	Amount (g/L)
Magnesium sulfate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bacto agar	15.0
Bacto brom thymol blue	0.08
Distill water	1 Litre

9. TSI

Ingredients	Amount (g/L)
Bio-polytone	20.0
Sodium chloride	5.0

Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous ammonium sulfate	0.2
Sodium thiosulfate	0.2
Phenol red	0.0125
Agar	13.0
pH	7.3
Distill water	1 Litre

10. MIU

Ingredients	**Amount (g/L)**
Proteose peptone	10.0
Beef extract	5.0
Sodium chloride	5.0
Dipotassium phosphate	2.0
Phenol Red	0.010
Agar	2.0
Distill water	1 Litre
pH	6.8

11. Sugar Fermentation Broth

Ingredients	**Amounts (g/L)**
Protease peptone	10

Beef Extract	1.0
Sodium Chloride	5.0
Dextrose/ Lactose/ Sucrose	5.0
Phenol Red	0.018
Distill Water	1 Litre
pH	7.4 ±0.2

12. T1N1 media

Ingredients	**Amount (g/L)**
Tryptone	10 g
Sodium chloride	10 g
Bacterial Agar	6 g
Distill water	1 Litre

APPENDIX – II

Reagents

1. **Kovacs Reagent**

1.25 g of para-dimethylaminobenzaldehyde was dissolved in 18.75 ml of amylalcohol. Then concentrated Hydrogen Chloride was added to make the final volume 25 ml. This reagent was covered with aluminum foil and stored at 4^{o}C.

2. **Methyl Red Reagent**

0.01 g of methyl red was dissolved in 30 ml of 95% ethanol. Then distilled water was added to make the final volume 50 ml. This reagent was covered with aluminum foil and stored at 4^{o}C.

3. **Barrits Reagent**

Solution A

1.25 g of alpha-naphthol was dissolved in 95% ethanol with constant stirring to make 25 ml solution. This solution was covered with aluminum foil and stored at 4^{o}C.

Solution B

10 g of Potassium Hydroxide (KOH) was dissolved in distilled water. The solution became warm. After cooling to room temperature, creatine was dissolved by stirring. Distilled water was added to adjust the final volume to 25 ml. This solution was covered with aluminum foil and stored at 4^{o}C

4. Oxidase reagent

100 mg of N, N, N^1, N^1-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10 ml of distilled water and covered with aluminum foil. Then the solution was stored at 4^oC.

5. Catalase Reagent

Hydrogen peroxide of 2 ml amount was dissolved in 18 ml distilled water and covered with aluminum foil. The solution was stored in 4^oC

Appendix III

Instruments

The important equipments used through the study are listed below:

- Autoclave, Model no: HL-42AE : Hirayama crop, Japan
- Sterilizer, Model no: NDS-600D : Japan
- Class II Microbiological safety cabinet : Labcaire, USA.
- Electric balance, Scout, SC4010 : USA
- Freezer (-30^{o}C) : Liebherr, Germany
- Refrigerator (4^{o}C) : Vest frost
- Incubator : Japan
- Micropipettes : Eppendorf, Germany
- Microwave oven, Model: D90N30 ATP : Butterfly, China