Assessing the amount of *Pseudomonas aeruginosa* and the amount of Heavy metals, Lead (Pb) and Chromium (Cr) from Household Water from different Industrial Zones of Dhaka Division (Hazaribagh and Savar)

By

Md Raihan Uddin
15126007
Sadia Ishrat
15226001

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

Microbiology Program,

Department of Mathematics and Natural Sciences,

Brac University

December 2019

Declaration

It is hereby declared that

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

Student's Full Name & Signature:

Md Raihan Uddin	**Sadia Ishrat**
15126007	15226001

Approval

The thesis/project titled, Assessing the amount of Pseudomonas aeruginosa and the amount of Heavy metals, Lead (Pb) and Chromium (Cr) from Household Water from different Industrial Zones of Dhaka Division (Hazaribagh and Savar), submitted by

1. Md Raihan Uddin (15126007)
2. Sadia Ishrat (15226001)

of Fall 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Science in Microbiology on 24/12/2019.

Examining Committee:

Supervisor:
(Member)

_____
Fahareen Binta Mosharraf
Senior Lecturer, Department of Mathematics and Natural Sciences, Microbiology Program,
Brac University

Program Coordinator:
(Member)

_____
Mahboob Hossain, PhD
Professor, Department of Mathematics and Natural Sciences,
Microbiology Program,
Brac University

Departmental Head:
(Chair)

_____
A F M Yusuf Haider, PhD
Professor and Chairperson, Department of Mathematics and Natural Sciences,
Brac University

Abstract

Water is the major natural reservoir of the atmosphere - not so much in volume as in time and contact. Water is the indispensable environment resource for human security, the driven force of engines of sustainable socioeconomic expansion. In recent days, due to presence of all types of living organisms and undesirable accumulation of heavy metals in water and their toxic impact on biological systems, the water quality is a concern of paramount prominence to ensure the health of the population around the world. The purpose of this study was to determine the quality of natural water including samples from reservoir and distribution by considering into the microbiological aspect and several physicochemical analyses. *Pseudomonas aeruginosa* is a bacterial species, which occurs widely in water environment capable of conferring wide range of diseases and have tendency to develop high antibiotic resistance. The magnitude of the heavy metals content of water may pose significant health risks so continuous monitoring of both chemical and biological quality of natural water sources is highly recommended. The literature cited here showed a parallel the assessment of the levels of the toxic chemicals like Lead (Pb) and Chromium (Cr) in two distinct locations, Hazaribagh and Savar, Dhaka city within the period of May2019- November 2019 along with the presence of *Pseudomonas aeruginosa*. Finally, the results portrayed that 20% of the samples from each area confirmed the presence of *P.aeruginosa* in the same sample as Lead that was present in 75% of the samples, whereas chromium was absent in all the samples.

Keywords: *Pseudomonas aeruginosa*: Heavy metals, Lead (Pb) and Chromium (Cr): Industrial zones: Household water: Dhaka Division

Acknowledgement

The piece of work we accomplished in pursuance of our B.Sc. dissertation happens to be the first undertaking of this nature we have ever been exposed to. It may be a small step as such but for us it was a great leap. We needed help and encouragement not to be frustrated in the event of repeated failures in our experiments. Fortunately, there were people around us who provided the needed supports.

Our regards, gratitude, indebtedness and appreciation goes to our respected supervisor **Fahareen Binta Mosharraf**, Senior Lecturer, Department of Mathematics and Natural Sciences, Microbiology program of BRAC University, for her constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and a good sense of humor and never ending inspiration throughout the entire period of our research work.

We are grateful to **Imon Rahman**, Department of Pharmacy of BRAC University, for his kind cooperation, active support and constant supervision.

We would like to thank and express our deepest gratitude to **Professor Saquiba Yesmine**, Department of Pharmacy, Jahangirnagar University, who helped and guided us in our heavy metal analysis that is carried out using AAS. It would have been impossible to submit our report without her cordial help.

We would also like to thank **Nilkhil Bhoumik**, Scientist at Wazed Miah Science Research Center, Jahangirnagar University for helping to operate machines related to our experiment.

We express our gratitude towards **Prof. Dr. A. F. M Yusuf Haider**, Chairperson, Department of Mathematics and Natural Sciences, BRAC University, for his kind cooperation, active support and constant supervision.

Our departmental teachers often enquired about our progress of the work and we were encouraged to keep in touch with them to have their valuable advice. We thank them all for their kind and affectionate care.

We would like to extend our special thanks to our seniors and batch mates in the laboratory, who provided us with good working environment by their advice and encouragement to make us feel at home in our hard times.

Our sincere appreciation is extended to staffs of the department, who helped us even beyond their duty period to continue our research work.

Md Raihan Uddin and Sadia Ishrat

December 2019

Table of Contents

List of Tables

List of Figures

List of Acronyms

^{0}C	Degree Celsius
min	Minute
sec	Second
h	Hour
d	Day
mg	Milligram
g	Gram
kg	Kilogram
L	Liter
ml	Milliliter
µL	Microliter
mM	Millimolar
mm	Millimeter
µm	Micrometer
e.g.	For example
et al.	And others
pH	Negative logarithm of hydrogen ion concentration
%	Percentage
UV	Ultra violet
spp.	Species
CFU	Colony forming unit

Chapter 1

Introduction

One of the eight components of primary health care is the allocation of sufficient and safe water. The fundamental dangers to human health by consuming contaminated water (WHO, Guidelines for Drinking-water quality: fourth edition incorporating the first addendum, 2017). It is used in almost all the activities, playing a vital role in the development of humans and other organisms, including all domestic purposes, such as, cooking, washing, bathing, drinking. However, safe water is not easily accessible for many people. Water pollution being an environmental hazard, is one of the leading worldwide cause of diseases. Countries undergoing industrialization continue to struggle with water pollution, counting more than 14,000 deaths daily.(Afrin, Mia, & Akter, 2015). While in Europe and America, clean and treated water is always available, in developing countries, people are exposed to unsafe water that eventually result in waterborne infections. Nearly 1.5 million children die each year from diarrhea and two and a half billion people have no access to improved sanitation (Fenwick A, 2006). Every year 5 million people are subjected to mortality due to water associated diseases (WHO, Surveillance and control of community supplies. Guidelines for drinking-water quality., 1997). The total earth's surface that is covered with water is 71% but out of this, the amount of fresh and safe usable water is only 2.5% (Shikhlomanov L A, 1993). Economic water shortage is now an issue for 1.6 billion people all over the world with two-thirds of the population face water scarcity in a year for at least a month (Mekonnen M M & Hoekstra A Y, 2016). Dhaka being one of the most populated cities in Bangladesh like the rest of the third world country, is addressing the same problem. Dhaka labelled as a mega city, houses a population of over 20 million people with an estimated growth rate of 3.62% according to the UN World Urbanization Prospects. Primarily Dhaka is dependent on the groundwater for the urban water supply drawing about 84% water from groundwater and 16% from surface water. (Hoque,

Hoque, & Ahmed, 2007). Despite the fact that 97% of the population have access to water assured by more than 230 rivers, the quality of the water is not up to a safe level because of the rivers being contaminated by pollution which are mostly caused by human activities (WHO, Sustainable Development and Healthy Environment., 2018) (Majumder A, 2009). It was reported by the Department of Environment of Bangladesh that surface water and river showed increased accumulated of pollution over a year of monitoring ground water and surface water (DoE, 2014).

The properties of water that are used to analyze water quality are physical, chemical and microbiological. Microbiological components includes bacteria and viruses that can possibly infect people causing diseases, such as, cholera, typhoid, bacillary dysentery, adenoviruses, retroviruses, and other diseases.

To narrow down microbiological analysis of water, *Pseudomonas aeruginosa* was the center of interest in this experiment. It is a rod-shaped aerobic bacteria with a single polar flagellum and is associated to the bacterial family Pseudomonadaceae and is a member of the Gamma Proteobacteria class (Novik G, 2015). Unlike fastidious organisms, it demands minimal necessities to survive in an environment (Leclerc H, 2002). It can use trivial amount (<100µg/L) and in a wide range of carbon sources but most preferably organic and fatty acids (van der Kooij D, 1982) as well as survive for months in distilled water or deionized water (Warburton D, 1994). It is a Gram- negative, aerobic bacteria that live primarily in soil, seawater and fresh water. It can easily grow and adapt in environments that are oligotrophic and also copiotrophic environments (Favero MS, 1971). Plants and animals are often known to be colonized by *P. aeruginosa* which enables them to be frequently found in homes and hospitals or clinical settings (Jefferies JMC, 2012). Different types of aquatic environments and a number of and wet moist places on plants and land are likely of interest to grow in (Hardalo C, 1997). Likewise, as for carbon as its energy source, nitrogen is also consumed and

obtained from variety of sources (Favero MS, 1971). To its favor, *P. aeruginosa*, is not obliged to use only oxygen as its electron acceptor. One of its pliable characteristics is its capacity to use nitrate as an electron acceptor and if neither one of them are present in the environment it can subsist by producing arginine or pyruvate (Schobert M, 2010). The optimal pH at which its growth will flourish is pH 7.2 but as for the pH range, it has not been precisely interpreted (Beyenal H, 2003).

As for the medium of transmission, water is considered to play a big role in augmentation and transmission of *P.aeruginosa*, although it is not agreeable concept for the field of medicines (Exner M, 2005); (Trautmann M, 2005); (Williams MM, 2013). Despite all statements of water being a suitable medium for *P.aeruginosa*, it is debatable since there are many other ways and possibilities for this organism to contaminate and infect patients or people, such as, patients directly or indirect may have been in contact with the source of pathogen. Nevertheless, effective cleaning of water systems shows significantly less amount of pathogens in water (Petignat C, 2006); (Rogues AM, 2007).

Another advantageous characteristics of *P.aeruginosa* is its ability to form biofilms, which is another important fact to consider since antibiotics cannot effectively treat infections caused by bacteria that can form biofilms (Rasamiravaka T, 2014). *P.aeruginosa* is an opportunistic pathogen for humans that leads to a wide spectrum of diseases that can be lethal and deadly for the infected person (Kerr KG, 2009). Extended period of time at the hospital increases the chances of being infected by *P.aeruginosa*. Infections, such as, septicemia, pneumonia, meningitis, malignant external otitis, endophthalmitis and endocarditis (Bodey GP, 1983). Thus, the hospitals at the industrial areas are at high risk of being infected by *P.aeruginosa*. Among these infections, the most note-worthy is ventilator-associated pneumonia (Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia., 2005). Next in the list comes gastrointestinal tract infections (not

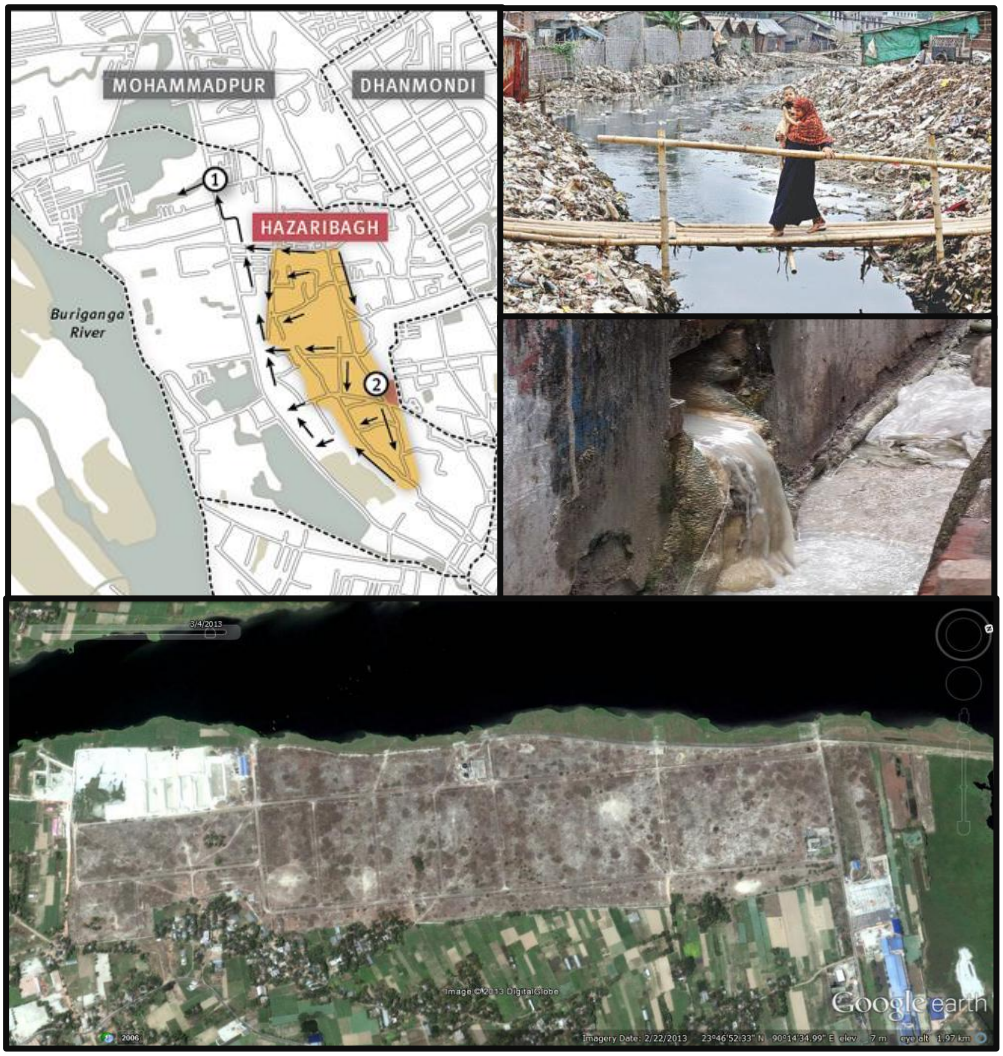
endoscopy-related) and catheter-related bloodstream infections (Wendy IS, 2015). By the same token, neonates and individuals with deep neutropenia poses a high risk at being infected (Jefferies JMC, 2012); (Kerr KG, 2009); (Leclerc H, 2002). It also targets immunocompromised patients such as post-surgery patients or patients from burn units that provides the pathogen an open wound to enter the body. It was examined that *P.aeruginosa* was found in 50%-60% in such patients and in 1.2-6% in healthy individuals (Botzenhart K, 1993). Other variety of infections includes urosepsis and wound infections (Berthelot P, 2006). Moreover, *P.aeruginosa* can colonize in the contact lenses and cause keratitis (Mena & Gerba, 2014). Convalescent from infections caused by *P.aeruginosa* seems to be difficult because treatment by antibiotics is getting out of the table due to its burgeoning resistant capacity (Keith P, 2005). Selective pressure is created on the pathogen when antibiotic is misused or abused that eventually produces mutant strains which are resistant to antibiotics. On the other hand, antibiotics unnecessarily destroy the good microbiome in our bodily systems that protects our systems from pathogenic bacteria and as well as reducing the competition for pathogens of source of energy (Rasamiravaka T, 2014).

Chemical components, to analyze water quality, can be organic or inorganic matter which could also cause health problems (Duressa G, 2019). One of such inorganic matters are heavy metals which are non-biodegradable and hence, harmful (Tilzer M M, 1993). Lower exposure to heavy metals can cause behavioral changes, effect on mind, abortion, cancer, kidney damage and even death on higher exposure (Saha P K & Hossain M D, 2011).

Despite adding more to economic growth and reducing poverty, expeditious industrialization at the current rate is one of the significant source of water contamination. This occurrence is due to the improper system of deposition of waste materials and biohazards released from the factories (Jolly Y N, 2013). Varieties of chemicals are used to treat the cow hides in order to get the finished products. Meanwhile, all the waste products such as sodium sulfate, sodium

hydroxide, sodium hydrosulfate, arsenic sulfide, chromium sulfate are dumped into the local drains. The variety of chemicals used by the tannery industries are considered to be the major contributor of heavy metals to the environment (Bhuiyan et al., 2011). As developing countries lack proper disposal techniques and have bad sewerage system, the heavy metals are being exposed to the rivers, animals, fishes and ultimately ending up as our food again. Not only the effluents discharges but also the fertilizers used by the farmers, fungicides along with all the other chemical runoffs are actively affecting the environment (Kibria, Hossain, Mallick, Lau, & Wu, 2016). In addition to that, industrial sectors in Dhaka show swift and unorganized growth resulting in damaged environment (The World Bank, 2018). Among the industrial sectors, textile and leather sectors are most responsible for deteriorating water quality, hence, are to be focused on as the source of contaminating tap water (Islam M R, 2013). Water samples from two locations were analyzed in this experiment, Hazaribagh (inside Dhaka) and Savar (on the outskirts of Dhaka). Both the areas were known to be populated with factories of different kinds. Recently, tannery industry were moved to Savar, an act intended to save the Buriganga River that is destroyed beyond repair (Strasser J, 2015). In Bangladesh Lead and Chromium, along with other metals are found in abundance from the surface water of the polluted rivers surrounding the capital (Chowdhury et al., 2016).

Figure 1: (Top left) The Hazaribagh tannery area (Cedillo A, 2012). (Top right) A woman with a child crossing a bridge from one disposal dumping site to another (Roy, 2019). (Middle right) Effluent discharge from a factories directly into water system (Kogure T, 2014). (Bottom) Relocation of tannery industries in Savar (Google Image, 2013)



A metallic element containing high density and is toxic even at low concentration is referred to as “heavy metals” (Leentech, 2004). Metals and metalloids with 5 time’s greater density than water and as well as atomic density greater than 4 g/cm^3 can be accumulatively called heavy metals (Hutton M & Symon C, 1986) (Garbarino J R, 1995). Contaminated water by heavy metals is used up by plants for photosynthesis which in turn is consumed by animals. In addition to that, marine lives growing in contaminated water collects heavy metals in their tissues. The accumulated heavy metals passes along the ecosystem inhabiting into the food chains of humans (Verma R & Dwivedi P, 2013). Heavy metal pollutants are very harmful, toxic and poisonous even in ppb (parts per billion) range (Rahman Z & Singh V P, 2019). The heavy metals that were analyzed in this experiment are Lead (Pb) and Chromium (Cr).

Sources of heavy metals (Gautam S P, 2013):

- Lead (Pb) – paints, lead acid batteries, smelting operations, E-waste, ceramic industry and power plants.
- Chromium (Cr) – Industrial coolants, manufacturing and leather tanning.

In general, a continuous exposure to toxic levels of heavy metals progressively leads to multiple sclerosis, Parkinson’s disease, and Alzheimer’s disease which are all neurological degenerative diseases. In addition to that, other physical and muscular damage may also occur. The result of the above conditions will consequentially lead to death (Verma R & Dwivedi P, 2013).

Effects of Lead and Chromium on Health:

1. Lead- The presence of lead in the body stimulates the process of DNA damage by disrupting the DNA repair system. Researchers have studied that this compound is responsible for altering chromosomal structure (Silbergeld EK, 2000). They cause

toxicity in ionic mechanism, as well as, in oxidative stress due to imbalance between free radicals and production of antioxidants, which in turns, disrupts cell repair mechanisms (Jaishankar M, 2014).

2. Chromium- *In vitro* studies explain that chromium causes free radicals to be produced from water which was detected in liver and blood during *in vivo* examination that results in oxidative stress (Liu KJ, 2001). In addition to that, it can also cause reproductive toxicity, embryo toxicity and teratogenicity. Such diseases are mostly caused by chromium(VI) Furthermore, chromium (VI) compounds are responsible for causing mutations in prokaryotic and eukaryotic organisms (WHO, Chromium in Drinking-water, 2003)

In addition to damaging human health, heavy metals hold the tendency to cause inhibition at various activity sites within the microorganisms, such as, blocking necessary functional groups. Besides inhibition, they are also able to dislocate metal ions essential for their metabolic activity and trigger the change in conformations of biological molecules from active form to inactive form. (Mittal S K & Ratra R K, 2000). However, the requirement of trace elements in metabolic reactions in an organism can be achieved from some of the heavy metals, such as, zinc, iron, copper and nickel in relatively low concentrations. Whereas, other heavy metals serve no purpose in metabolic reactions, rather, are damaging to the organisms even at low concentrations (Gadd G M & Griffiths A J, 1977-1978). This includes mercury, cadmium and silver. These heavy metals are toxic to microorganisms. However, over the years, some bacteria developed strains that allows them to survive in the presence of heavy metals. Development of such resistance is equivalent to the resistance mechanism to antibiotics. As a matter of fact, the metal and antibiotic resistance genes are located on the same mobile genetic elements. Inevitably, heavy metals resistance is caused due to selective pressure (Silva A A L, 2012).

Chapter 2

Materials and Methods

2.1 Working laboratory

The first phase of the research that included both enrichment and isolation of *P. aeruginosa* from water samples was performed in the Microbiology Specialized Research Laboratory, Department of Mathematics and Natural Sciences, BRAC University.

The second phase of the research that incorporated determining the presence and level of heavy metals in water samples was performed in the Wazed Miah Science Research Centre, Jahangirnagar University.

2.2. Water source

In this research, 250 ml of 20 polypropylene bottles were used to collect from Savar and 20 water samples from Hazaribagh in Dhaka. The locations of the collected water samples are tabled in Table 1 and Table 2.

Table 1: Location of Sample Collection from Savar

Sample ID	**Location**
F1	Moddo Para Minar Masjid O Kashemoul Uloom Madrasha
F2	Jhawchar Bazar
F3	Bongaon Union
F4	Savar thana road
F5	Hemayetpur, beside Adhunik Plastic Industry
F6	Baliarpur
F7	Baipayl
F8	Beside Enam Medical College
F9	Ulail Bazar
F10	Gam Factory
F11	Hemayatpur Bazar
F12	Jamgara
F13	Radio Colony Bus Stand
F14	Jahangirnagar University Central Mosque
F15	Savar Bazar, Shahjalal Market
F16	Gono BishwaBidalay
F17	Jahangirnagar Bot tola
F18	Bismail
F19	Amin Bazar
F20	Ideal Bazar

Table 2: Locations of Sample Collection from Hazaribagh

Sample ID	Location
B1	Baitul Muharram Jame Mosque
B2	Tannery Block Jame Mosque
C1	Near Asia Trading Corporation ltd.
C2	Near Sikder Medical Bus Stand
C3	Tannery Mor Shahi Masjid
D1	Gojmohal Boro Mosjid
D2	Ambia Jame Masjid
D3	Nobipur Jame Masjid
E1	Dhaka Tannery mor
E2	Hazaribag Kacha bazar
E3	Borhanpur Jame Masjid
E4	Mitali Bazar
E5	Moneshwar road
E6	Hazaribag Bazar
E7	Hakimunnesa Jame Masjid
E8	Talli Office Road
E9	Sadar Ghat Gabtoli road
E10	Masjidul Aqsa Jame Masjid & Madrasha
E11	Tea stall at Sher e bangla Road
E12	Asian Tannery Mosque

2.3 Weather forecast

Table 3: Weather of the day of Sample Collection

Location	Temperature	Humidity	Precipitation	Wind flow
Savar	30℃, Cloudy	60%	23%	9 km/h
Hazaribagh	31℃, Cloudy	67%	20%	6 km/h

2.4 Bacterial specimen

Pseudomonas aeruginosa

2.5 Types of Agar Media

Nutrient Agar is composed of beef extract, peptone and agar which allows the growth of non-fastidious organism serving as a general-purpose medium. Here the beef extract provides carbohydrates, vitamins, organic nitrogen compounds, though the peptone is the primary source of organic nitrogen. And the complex carbohydrate agar helps the medium to solidify at a 45ºC temperature.

Cetrimide Agar, composed of pancreatic digest of gelatin, magnesium chloride, Cetyltrimethylammonium Bromide, Glycerine, Potassium sulfate and Agar, is both a selective agar and a differential agar for the isolation of pseudomonas aeruginosa. Cetyltrimethylammonium Bromide acts as a cationic detergent and inhibits other bacteria. In the presence of the toxin, pyocyanin produced by P. aeruginosa combines with another toxin called pyoverdine and produces the bright green color colonies exhibiting the presence of P. aeruginosa.

Tryptone Soya Broth, TSB was used as an enrichment media to enhance the growth of the organism present in the samples. The pancreatic digest of casein, papaic digest of soyabean meal, dibasic potassium phosphate along with dextrose and sodium chloride in TSB composed a well-balanced nutrition for the organism to grow and increase the microbial load for better isolation.

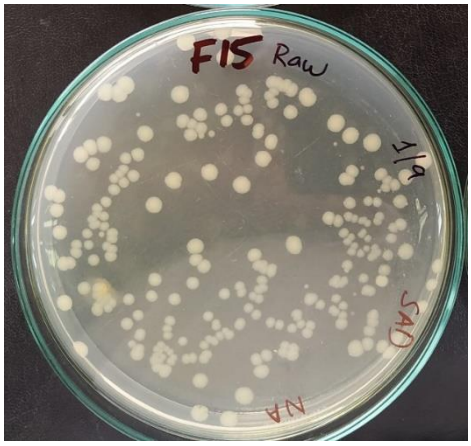
2.5.1 Preparation of plating bacteria on Nutrient agar medium

1. On a Petri dish containing nutrient agar medium, raw water sample was plated using spread plate method. The same water sample was then diluted into dilution factors 10^{-1} and 10^{-2}. Using spread plate method again, the diluted samples were inoculated into petri dish for each dilution.

2. The petri dishes were incubated at 37ºC in an incubator for 48hours.

   After incubation, the CFU per ml was counted for both the raw and diluted samples and the readings were recorded.

Figure 2.1: Growth of Organism on Nutrient Agar



2.5.2 Morphology and Identification

Belonging to a Gram-negative group of organisms, Pseudomonas aeruginosa is aerobic, rod shaped are 0.5-1.0 µm by 1.5 – 5.0µm in size. Often associated with human infection, these microorganisms have a very strict aerobic respiratory metabolism and grow between temperatures 5 to 42ºC. The characteristic blue green appearance on the culture and the grape like smell of aminoacetophenone is indicative of the presence of the organism. The colorful appearance is due to the mixture of pyocyanin (blue) and pyoverdin (fluorescein, yellow). They require an incubation of 24 hours at 37ºC and can exhibit multiple type of colonies. Moreover, the *P. aeruginosa* isolated from the cystic fibrosis patients needs a variant type of incubation. The incubation period extends to 48 hours and the temperature lowers to 30ºC in order to obtain proper phenotypic characteristics. These mono flagellum organisms may appear large, rough, convex, wrinkled boarders and mucoid (NHS, 2015).

2.5.3 Gram-Staining

It is a technique that distinguishes between gram-positive and gram-negative organisms based on their outer cell membrane constituent. Bacterial colonies were taken from Nutrient agar and made a heat fixed smear on a microscopic slide. A primary stain (Crystal Violet) was added over the smear and kept for 45 seconds. After washing under running water, a mordant (Iodine) was added over the smear for 30 seconds. Alcohol is used as a decolorizing agent. Then, counter stain (Safranin) was added for 1 minute.

On observing the slide under microscope, Gram-positive bacteria appears as bluish-purple and Gram-negative as red.

Figure 2.2: Gram Positive Organism appear Bluish-purple under microscope

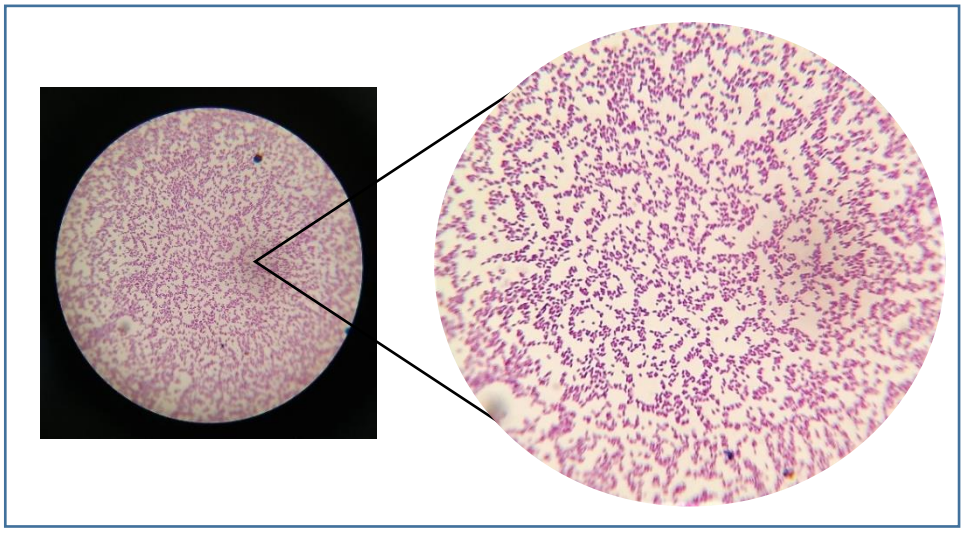
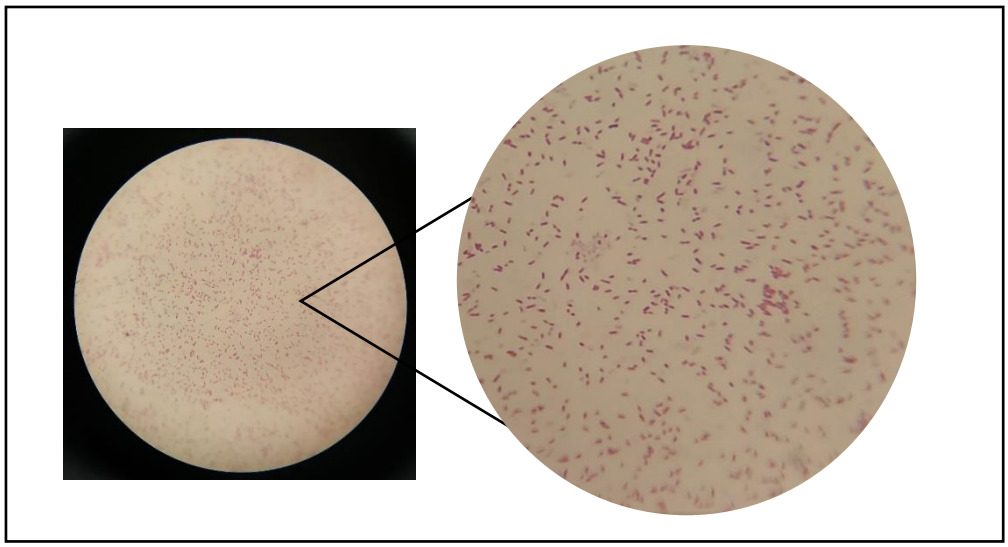


Figure 2.3: Gram Negative Organism appear Pinkish-red under microscope.



2.5.4 Preparation of plating bacteria on Cetrimide agar medium

1. Raw samples were inoculated in a Petri dish containing Cetrimide agar medium, using spread plate method.

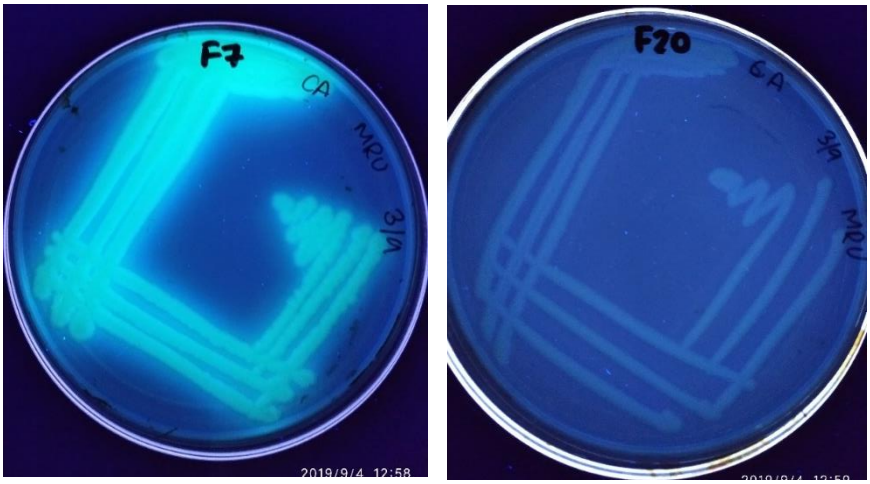
2. The petri dishes were incubated at 37^{o}C in an incubator for 48 hours.

3. After incubation, the growth was observed under UV light to detect the presence of glowing colonies.

2.5.5 Confirmation of *Pseudomonas aeruginosa* on Cetrimide agar medium

A good number of *Pseudomonas aeruginosa* could be inferred from many of the water samples as it produced green yellow glowing colonies under UV light on cetrimide agar due to the presence of pyocyanin.

Figure 2.4: Glowing and non-glowing colonies under UV light on a Cetrimide Agar.

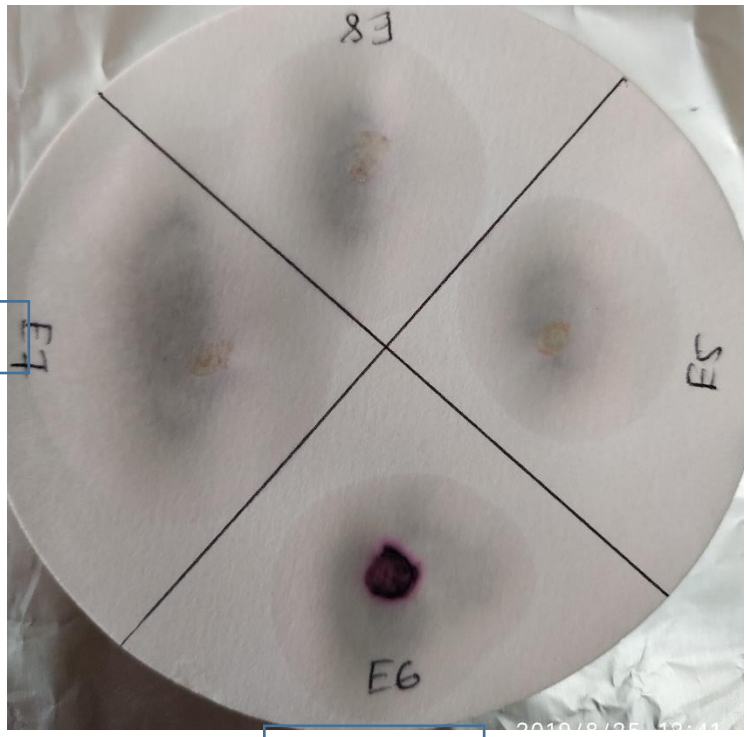


2.6 Biochemical Identification

1. Oxidase test-

    - A loopful of bacteria from Nutrient agar plate was streaked onto a piece of filter paper (Whatman, 1MM).
    - A few drops of oxidase reagent were added onto the streaked bacteria on the filter paper. Positive reactions turned the bacteria from violet to purple within 1 to 30 seconds. Delayed reactions should be ignored.

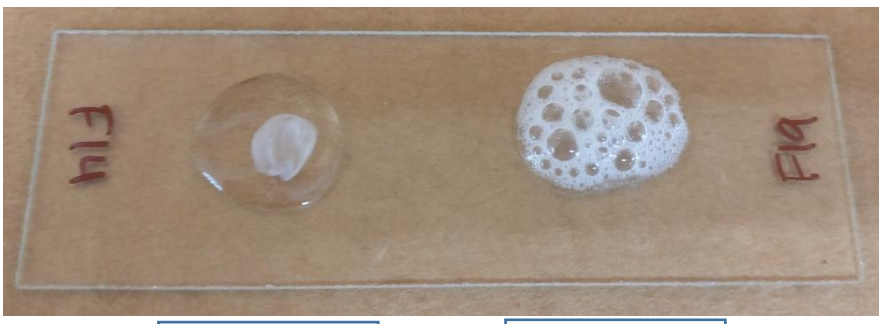
Figure 2.5: Oxidase Test



2. Catalase test-

- The test detects the presence of catalase enzyme that breaks down hydrogen peroxide into oxygen and water. Bacterial colonies were taken from Nutrient plate and using a loop transferred on to a microscopic slide.
- Positive result shows formation of gas denoted by bubbles. Negative result shows no change.

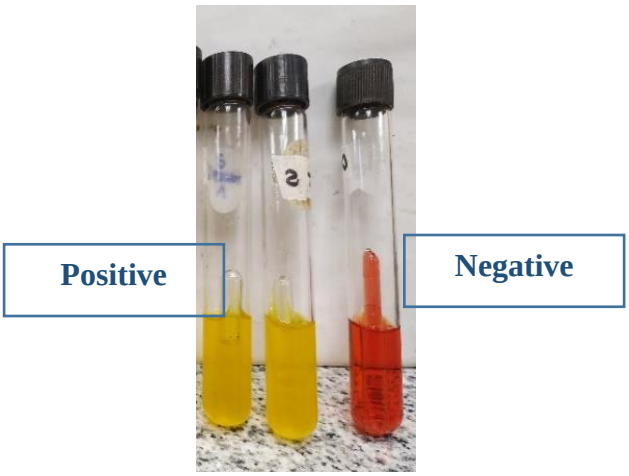
Figure 2.6: Catalase Test



3. Dextrose fermentation-

- The tubes were aseptically inoculated with the bacterial colonies from the freshly prepared nutrient agar plates into the phenol red dextrose broth, using an inoculating loop and were incubated for 24 hours at 37ºC.
- After incubation, on alteration of the color of the liquid of the tube to yellow would indicate a positive change, i.e. the organism produced acid which turned the solution yellow, indicated by the pH indicator phenol red. But if the liquid remained red, indicates a negative result, no acid formation and no dextrose fermentation occurred.

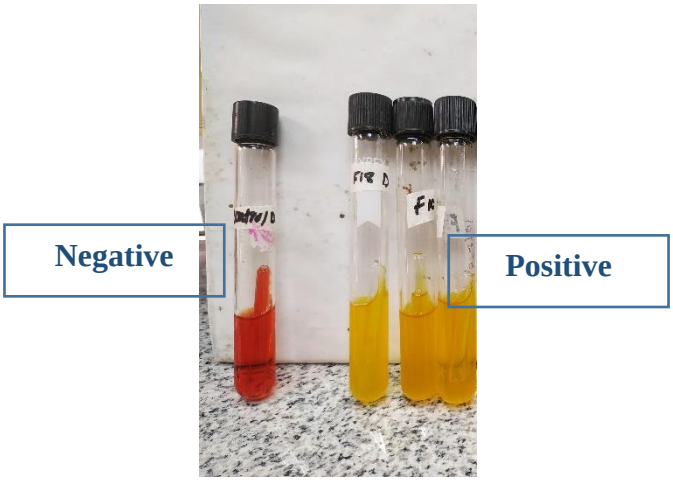
***Figure 2.7*:** Dextrose Fermentation



4. Sucrose fermentation-

- Phenol red sucrose broth were aseptically inoculated with the bacterial colonies from the nutrient agar plates and incubated for 24 hours at 37ºC.
- The alteration of the color of the broth to yellow would indicate acid production from the fermentation of the carbohydrate, sucrose. If the broth remained unchanged, it would indicate no fermentation.

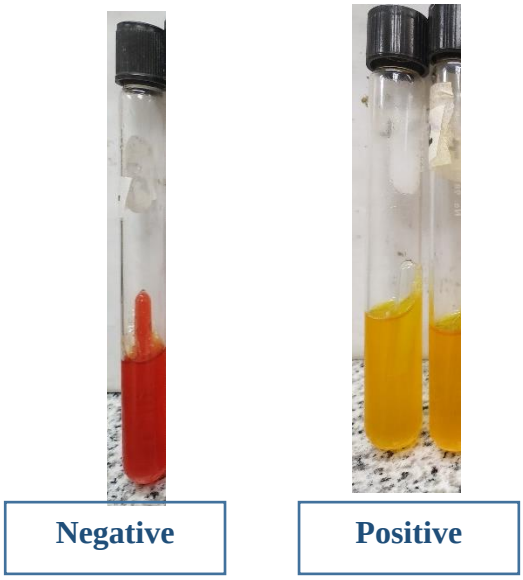
***Figure 2.8*:** Sucrose Fermentation



5. Lactose fermentation-

- The bacterial colonies were picked using an inoculating loop from the fresh nutrient agar plates and were inoculated into the phenol red lactose broth and left for incubation at 37ºC overnight.
- After 24 hours of incubation, if the color of the broth turned yellow it would indicate a positive result, because of the pH indicator present in the broth. With no acid production, the negative result would indicate no color change, proving the organism cannot ferment lactose.

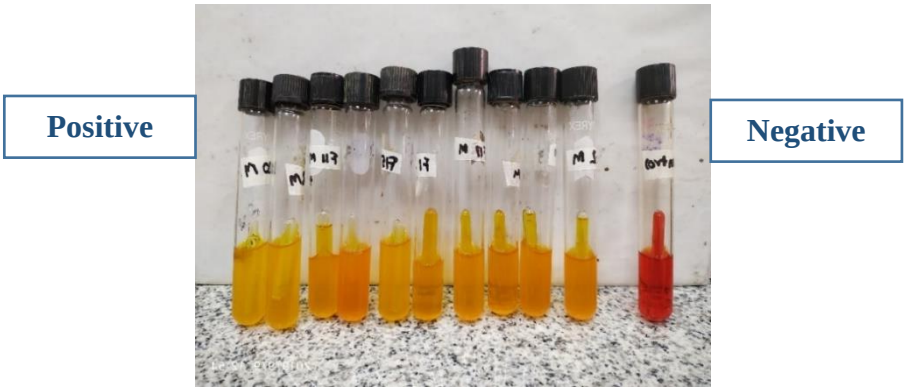
Figure 2.9: Lactose Fermentation



7. Mannitol fermentation-

- Phenol red mannitol broth was used to observe the fermentation of mannitol, where phenol red is the indicator of the pH change. Tubes of the broth were inoculated with the bacteria from the nutrient agar and incubated for 24 hours at 37ºC.
- Mannitol fermentation would be indicated with the color change of the broths. If it turned yellow from red, a positive result would have produced. If no fermentation occurs, the broth would remain red as it is even after incubation.

***Figure 2.10*:** Mannitol Fermentation

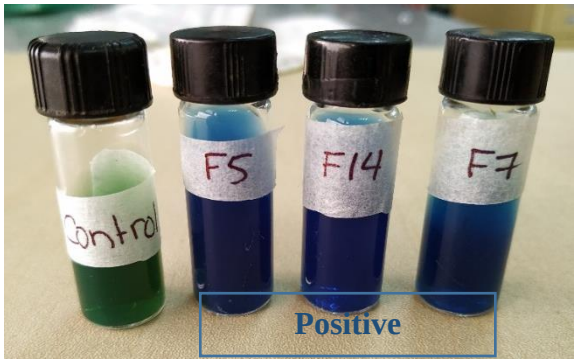


6. Citrate utilization-

Bacterial colonies were picked up from Nutrient agar plate by a straight wire and inoculated into the slope of Simmons' citrate agar and incubated overnight at 37ºC.

If the organism had the ability to utilize citrate, the medium changed its color from green to Prussian blue; a negative slant would have no growth of bacteria and would remain green.

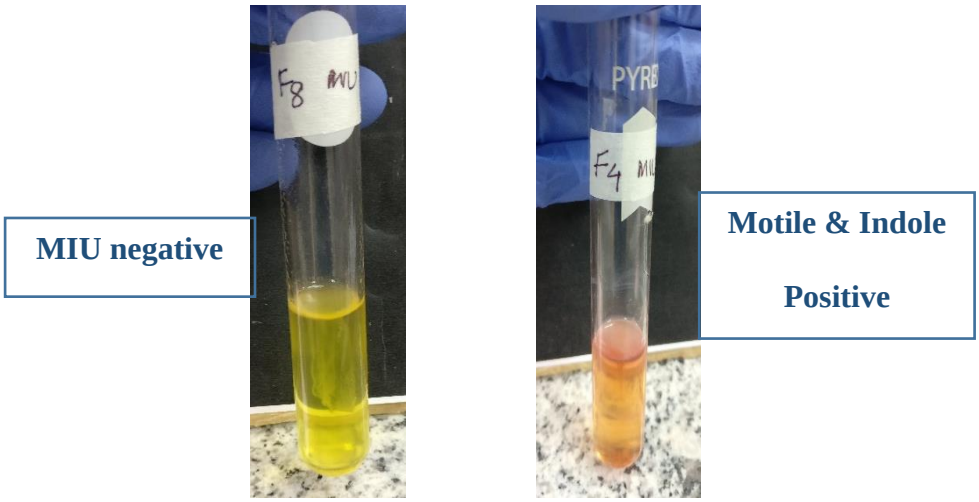
***Figure 2.11*:** Citrate Utilization



8. MIU test-

- This test observes the motility of the organism along with its ability to produce indole and breakdown urea to detect the presence of urease. Bacterial colonies were taken from Nutrient agar plate and inoculated into semi-solid medium.
- Motility is positive if the organism shows growth that diffuses away from the stab-line. Urease is positive if the color changes from yellow to pink. Absence of urease shows no color change. Production of indole is presented by the formation of a cherry-red ring at the top of the tube and negative if yellow or brown color change.

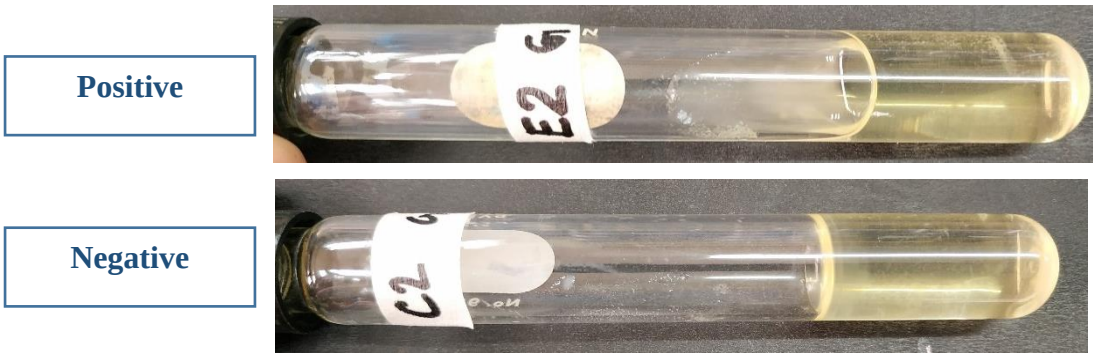
***Figure 2.12*:** MIU test



9. Gelatin hydrolysis-

- This test is used to examine the ability of an organism to produce gelatinases that hydrolyze gelatin. Bacterial colonies were taken from Nutrient plate and inoculated into test tube containing medium with gelatin with a straight wire.
- Presence of gelatinase enzyme shows partial or complete liquefaction of the medium. Absence of gelatinase shows no liquefaction.

***Figure 2.13*:** Gelatin Hydrolysis

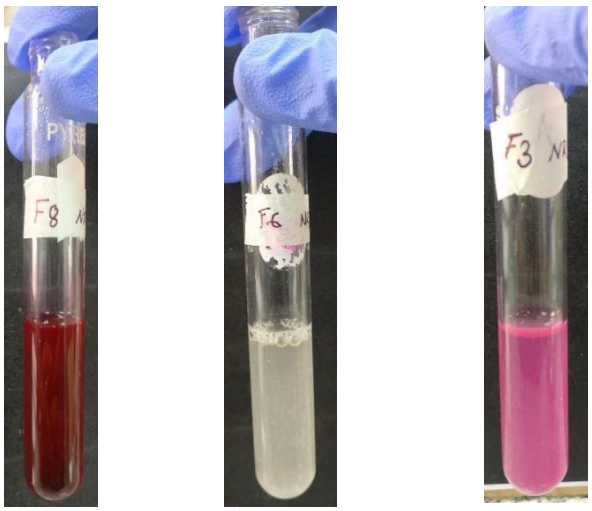


10. Nitrate Reduction-

This test determines the production of an enzyme called nitrate reductase, which results in the reduction of nitrate. Bacterial colonies were taken from Nutrient agar plate and inoculated into medium with a loop. After incubation, 6-8 drops of nitrite reagent A and add 6-8 drops of nitrite reagent B was added.

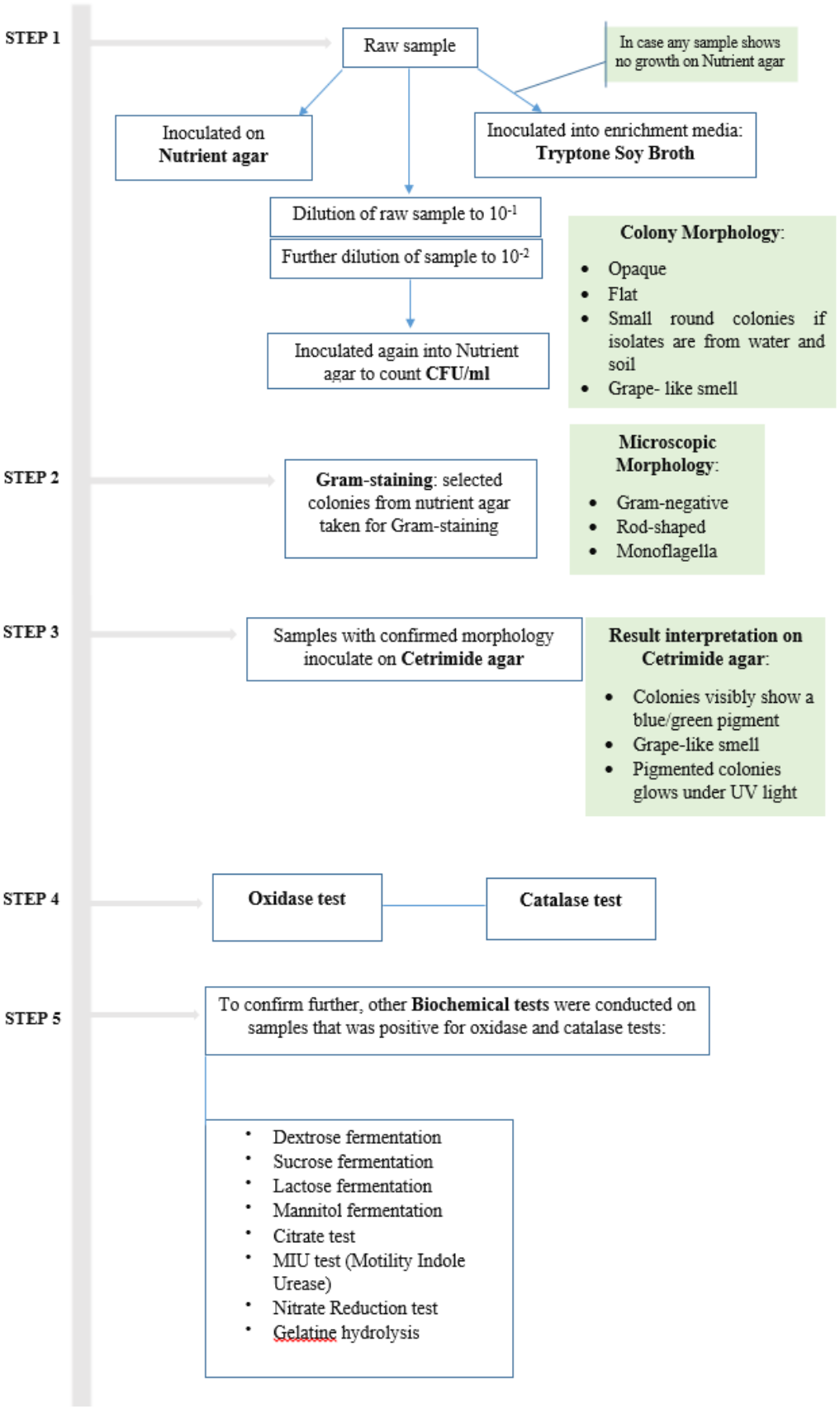
Partial positive result is indicated by a red color change on addition of the reagents. If no color change occurs, zinc powder is added. If yet shows no color change, the result is complete positive. If it turns pink, the result is negative.

***Figure 2.14*:** Nitrate Reduction: (a) Partial positive, (b) Full positive and (c) Negative



(a) (b) (c)

***Table 4*:** Flowchart of the Process of isolation of *Pseudomonas aeruginosa*



2.6.1 Probable Biochemical test results for *Pseudomonas aeruginosa*

***Table 5*:** Confirmatory Results by *Pseudomonas aeruginosa* (King A & Phillips I, 1978)

BIOCHEMICAL TESTS	RESULTS FOR *Pseudomonas aeruginosa*
Dextrose fermentation	Negative
Lactose fermentation	Negative
Sucrose fermentation	Negative
Citrate	Positive
Mannitol fermentation	Positive
Motility	Positive
Urease	Negative
Indole	Negative
Gelatin hydrolysis	Positive
Catalase	Positive
Oxidase	Positive
Nitrate reduction	Positive
Gram staining	Gram-negative, Rod shaped

2.7 Preparation of stock sample

2 ml of T_1N_1 agar was prepared in the ratio of 1:1:2 of tryptone, sodium chloride and agar in a vial and it was inoculated by taking pure bacterial colony of each isolate from nutrient agar plate. Then the vial was incubated at 37^{o}C for 24 hours. After incubation, the surface of the medium was covered with sterile autoclaved paraffin oil and the vial was stored at room temperature.

2.8 Heavy metals examined in the water samples

Lead (Pb) and Chromium (Cr)

2.8.1 Heavy metals analysis by Atomic Absorption Spectrometer

This procedure requires the use of atomic absorption spectrometer to determine the presence and concentration of analytes present in the sample. The calculation of the concentration of heavy metals were carried out in Wazed Miah Science Research Center of Jahangirnagar University using their Atomic Absorption Spectrophotometer (AAS) (Shimadzo, AA7000, Japan). This machine is highly sensitive and is used to run analysis for a large number of metals viz, Lead, Cadmium, Chromium, Selenium, and Nickel.

It includes atomizing the analytes in the sample so that they can be analyzed. Two types of gases are required for the flame to ignite and keep it burning. Acetylene was used as the fuel and nitrous oxide as the oxidant. Most common atomizers are flames and electrothermal (graphite tube). Using optical radiation, the atoms are then irradiated. The irradiated atoms pass through a monochromator in order to be separated from other radiations. This separation is detected and measured by a detector.

Figure 2.15: Atomic Absorption Spectrophotometer (Shimadzo, AA7000)

2.8.2 Acid digestion of water samples before running AAS

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

***Figure 2.16*:** Acid Digestion using Nitric Acid and Hydrochloric Acid

2.8.3 Permissible levels of heavy metals (WHO, Guidelines for Drinking-water quality: fourth edition incorporating the first addendum, 2017)

***Table 6*:** Permissible levels of Heavy Metals

	Heavy metal	**WHO guideline value/ ppm**
1.	Lead (Pb)	0.01
2.	Chromium (Cr)	0.05

Chapter 3

Result and Discussion

A total of 40 tap water samples were collected from Hazaribagh and Savar industrial areas, 20 samples from each area.

3.1 Isolation of *Pseudomonas aeruginosa*

The following tables shows the result of culture media and Gram-staining tests that were conducted at the beginning of the experiment, in order to isolate and determine the presence of *Pseudomonas aeruginosa* (King A & Phillips I, 1978) (Public Health England, 2015) (Siegrist J, 2007).

Table 7: Test results of samples from **Hazaribagh**

		Samples							
	Tests	**B2**	**C3**	**D2**	**E2**	**E4**	**E6**	**E7**	**E9**
1	**TSB**	+	+	+	+	+	+	+	+
2	**Nutrient agar**	+	+	+	+	+	+	+	+
4	**CFU/ml**	$7x10^3$	$1.6x10^3$	$5x10^3$	$6x10^3$	$2x10^3$	$9x10^2$	$1.3x10^2$	$5.7x10^4$
5	**Gram-staining**	Gram - negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative
6	**Cetrimide agar**	+	+	+	+	+	+	+	+
7.	**Oxidase**	+	+	+	+	+	+	+	+
8.	**Catalase**	+	+	+	+	+	+	+	-

Table 8: Test results of samples from **Savar**

		Samples						
	Tests	**F1**	**F4**	**F7**	**F9**	**F12**	**F14**	**F18**
1	**TSB**	+	+	+	+	+	+	+
2	**Nutrient agar**	+	+	+	+	+	+	+
4	**CFU/ml**	$9x10^2$	$6.1x10^2$	$5.3x10^2$	$5.2x10^2$	$7x10^2$	$2.3x10^2$	$4.5x10^2$
5	**Gram-staining**	Gram - negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative
6	**Cetrimide agar**	+	+	+	+	+	+	+
7.	**Oxidase**	+	+	+	+	-	-	-
8.	**Catalase**	-	+	+	+	+	-	+

3.2 Discussion of Table 7 & 8

Among 20 samples from each area, 8 samples from Hazaribagh and 7 samples from Savar were observed to have given opaque, small round colonies from isolated growth in Nutrient agar, along with grape-like odor because of the presence of aminoacetophenone. Colonies that showed the appropriate morphology on Nutrient agar were prepared next for Gram-staining. The samples that confirmed to be Gram-negative was due to the observed color of the organism which was pinkish-red in appearance and rod-shaped. Blue/purple colonies were not carried forward for Cetrimide test. On Cetrimide agar, the appearance of the colonies were observed to have a bright greenish appearance and on further observation in the UV light, the bright greenish appearance gave a glow of greenish/ bluish color. This color is caused due to the presence of pyocyanin (blue) and pyoverdin (green). Production of blue-green pigment is

indicative of *P. aeruginosa* (Public Health England, 2015). *E.coli* can also grow on Cetrimide agar, but gives no glowing colonies, thereby, distinguishing itself from *P.aeruginosa.* Another indication was the positive result from oxidase and catalase test. *P.aeruginosa* has the enzymes catalase and cytochrome c oxidase. Catalase converts hydrogen peroxide to oxygen and water and cytochrome C oxidase being the enzyme working at the terminal of respiratory chain reaction. Conducting these two tests meant elimination of sample E9 from Hazaribagh area and samples F1, F12, F14 and F18 from Savar area.

Although the above tests are usually conducted as the basic tests for *P.aeruginosa* (Public Health England, 2015) for determining the presence of the organism in a sample, it is wise to further carry out more biochemical tests. Growth on Nutrient agar, glowing colonies on Cetrimide agar and Gram-staining quite approves of the presence of *P.aeruginosa* (Brown V I & Lowbury J L, 1965). It specifically indicates the morphological match that includes appearance of colonies and observation stained bacteria under the microscope. However, it is wise to further carry out other biochemical tests to confirm (King A & Phillips I, 1978) (Public Health England, 2015) (Siegrist J, 2007).

3.3 Further Biochemical Tests to Confirm *Pseudomonas aeruginosa*

Table 9: Biochemical Test Results

	Tests	Confirmatory result for *P.aeruginosa*	Samples									
			Hazaribagh							Savar		
			B2	C3	D2	E2	E4	E6	E7	F4	F7	F9
1.	**Dextrose fermentation**	Negative	–	–	–	+	–	–	+	–	–	–
2.	**Sucrose fermentation**	Negative	–	–	–	+	+	–	–	–	–	–
3.	**Lactose fermentation**	Negative	–	–	–	+	–	–	+	–	–	+
4.	**Mannitol fermentation**	Positive	–	+	–	–	+	+	–	+	+	–
5.	**Simmons' Citrate test**	Positive	+	+	–	–	+	+	–	+	+	–
6.	**Motility test**	Positive	–	+	–	+	–	+	+	+	+	+
7.	**Indole test**	Negative	+	–	+	–	+	–	–	–	–	+
8.	**Urease test**	Negative	–	–	–	+	–	–	–	–	–	+
9.	**Gelatin hydrolysis**	Positive	+	+	+	+	+	+	+	+	+	+
10.	**Nitrate Reduction**	Positive	+	+	+	–	+	+	+	+	+	–

3.4 Discussion of Table 9

Despite of the fact that biochemical tests are used to accurately identify or isolate an organism and are also widely used for this purpose in many hospital laboratories, it is possible that some of the tests conducted may result to be a false positive or false negative. This in accuracy may be caused due to inaccurate measurements taken during preparation of the experiments, flawed reagents or experimenter's error. Therefore, all of these experiments were repeated twice to eliminate chances of inaccuracy. In addition to that, this experiment will be extended forward for conducting PCR on the samples.

From the biochemical tests, the samples that show precise results were C3 and E6 from Hazaribagh and F4 and F7 from Savar, confirming the presence of *P.aeruginosa.*

3.5 Heterotrophic Plate Count

Microorganisms that require organic carbon for proliferation are called Heterotrophs. Heterotrophic Plate Count Measurements are not exactly universal. However, some methods are standardized. HPC holds a long history in analyzing water quality in terms of microorganisms.

Table 10: HPC Acceptable Limit (Public Health Ontario, 2019)

Samples	CFU/ml	HPC Acceptable limit/ ml	Remarks
C3	$1.6x\ 10^3$	<500	About 3 times more than the acceptable limit.
E6	$9x10^2$		About 2 times more than the acceptable limit.
F4	$6.1x10^2$		About 1.5 times more than the acceptable limit.
F7	$5.3x10^2$		About 1.05 times more than the acceptable limit.

3.6 Discussion of Table 10

Samples that confirmed for *P.aeruginosa* showed CFU/ml to be exceeding the HPC acceptable limit. This indicates that the water is not safe for drinking or using for household purpose. Utilizing this water may result in skin, eyes or ear infections. On long term exposure will cause lung diseases and gastrointestinal problems (Mena KD & Gerba CP, 2009).

3.7 Relation between *Pseudomonas aeruginosa* and Heavy metals

Subsequently, these 4 samples were proceeded to be analyzed for heavy metals. This evaluation was conducted to observe the survival capacity of *P.aeruginosa* in the presence of heavy metals. Surface and underground water sources are often polluted by heavy metals resulting in considerable unsafe availability of water (Duruibe JO, 2007). Unplanned industrialization and its prompt growth are much accountable for allowing heavy metals contamination in water due to its improper discharge of effluents and waste products (Belal AR, 2015).

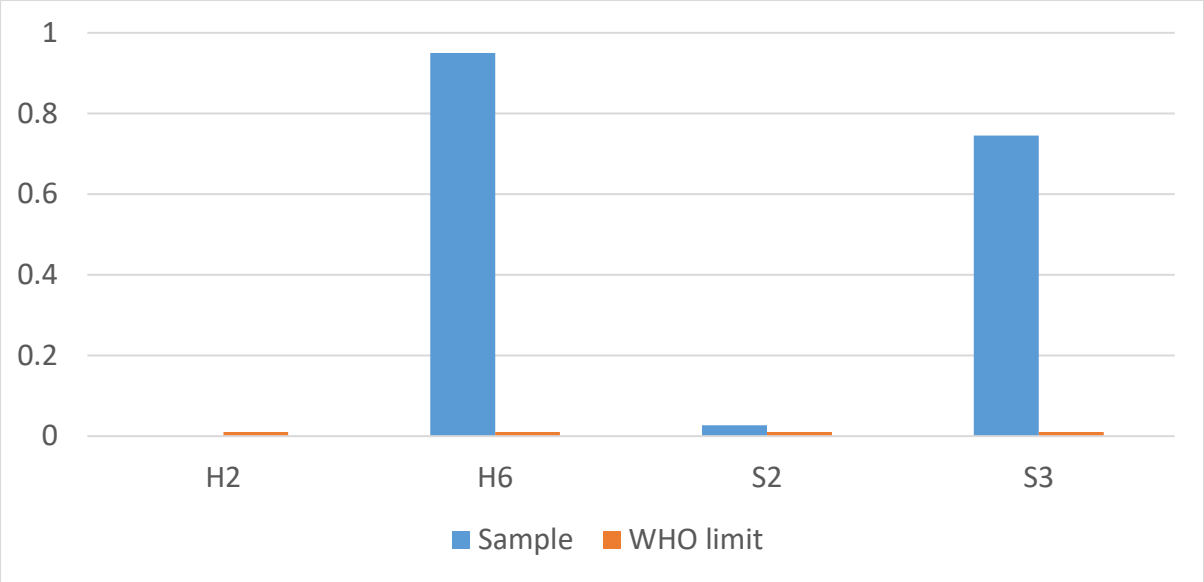
The soil or land around industrial areas are contaminated from the waste effluents from the factories, allowing the release of heavy metals into the water sources that supplies water through tap to different houses around the industrial areas. Health hazards caused by toxic levels of heavy metals can be responsible for central nervous function breakdown, lung, kidney and liver damage (WHO, Guidelines for Drinking-water quality: fourth edition incorporating the first addendum, 2017).

The study showed the concentrations of lead (Pb) and chromium (Cr) obtained from tap water in Hazaribagh and Savar (Industrial areas) used for cleaning and other domestic chores in the local area. Concentrations of the heavy metals studied are presented in Table 11.

Table11: Heavy metal concentration as general values, averages and WHO limit (WHO, Guidelines for Drinking-water quality: fourth edition incorporating the first addendum, 2017).

Location	Metals	Sample	Concentration (ppm)	Mean± SD	WHO limit (ppm)
Hazaribagh	***Lead (Pb)***	C3	0 (-0.015) 0.021 0 (-0.031) 0	0 (-0.006±0.022)	0.010
		E6	0.945 0.935 0.971	0.950±0.018	
	Chromium (Cr)	C3	0 (-0.007) 0 (-0.008) 0 (-0.011) 0 (-0.006)	0 (-0.008±0.002)	0.05
		E6	0 (-0.009) 0 (-0.003) 0.000 0.001	0 (-0.003±0.004)	
Savar	***Lead (Pb)***	F4	0 (-0.020) 0.036 0.046 0.046	0.027±0.031	0.010
		F7	0.781 0.724 0.729	0.745±0.031	
	Chromium (Cr)	F4	0 (-0.003) 0 (-0.002) 0 (-0.002)	0 (-0.003±0.000)	0.05
		F7	0 (-0.008) 0 (-0.007) 0 (-0.006)	0 (-0.007±0.000)	

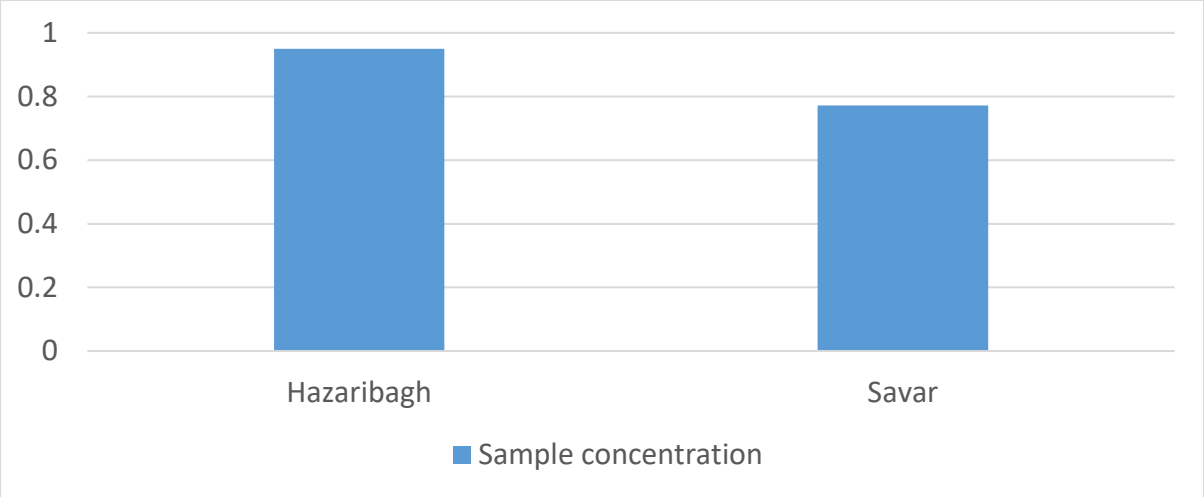
Figure 3.1: Concentration of Lead as compared to WHO limit



Lead- All the samples, except C3, were observed to have heavy metal content that exceeded WHO limit from both the areas. This indicates that the water released from industrial areas which likely consists of improper disposal of acid lead batteries and other chemicals, might be the source of lead pollution in Hazaribagh and Savar tap water source. One of the most trivial toxicant source of heavy metal is lead and it can be effortlessly absorbed by the body through food, water and inhalation (Rajkovic MB, 2008).

Chromium- None of the samples examined has chromium present in them. Therefore, it is safe to say the areas are free from Chromium compounds and the people from the areas will not be affected from illnesses caused by chromium.

***Figure 3.2*:** Comparison of Lead concentration (ppm) of Hazaribagh and Savar



On comparing lead data of Hazaribagh with Savar it was observed that Hazaribagh contains greater amount of lead than Savar and hence, the people are at more risk of being diseased by lead toxicity.

The importance of safe and quality water makes a prolonged list of activities that are required for human survival and also building of their lives. Assurance of safe and quality water cannot always be granted due to the lack of proper waste disposal systems and the increasing urbanization that only leads to producing more wastes, that in turns, causes water pollution (Ivy N, 2015). This experiment analyzed heavy metal content of water, precisely, Lead (Pb) and Chromium (Cr) using Atomic Absorption Spectrometer. From the previous experiment, the samples that showed the presence of *Pseudomonas aeruginosa* was then used for heavy metal analysis. Heavy metals are also toxic to microorganisms. However, bacteria, such as, *P.aeruginosa* have developed a resistance stain over the years and hence, their increase rate of survival in water with heavy metals (Haroun AA, 2017).

Evaluation of sample C3, E6 from Hazaribagh and F4, F7 from Savar indicated the presence of *P.aeruginosa*, the HPC of which exceeded the acceptance limit. On the same account, these

samples also showed higher amount of lead present in them (except C3). This determines that *P.aeruginosa* has the capacity to grow in the presence of lead.

Possibility of lead causing damage to this organism by blocking necessary functional groups has been eliminated along with other activities that lead may have caused in the organism to disrupt its metabolic activities (Mittal S K & Ratra R K, 2000). Survival in the presence of lead was most likely conceivable by making itself resistant to lead in the same mechanism as resistance to antibiotics happens. (Silva A A L, 2012).

Chapter 4

Conclusion

Water is the basic element of life. Depending on the different lifestyles, climatic conditions, traditions, cultures and diet, people use water for various purpose, such as, drinking, cooking, manufacturing goods purpose, agriculture, producing energy and so on (United Nations, 1977). Much to regret, adequate safe and clean water remains unavailable to world's huge population, precisely for people in developing countries. Recent studies predict that more than 1.7 billion people have no access to adequate sanitation services in 1990, while over 1.2 billion people lacked adequate clean drinking water (Gleick, 1993). This experiment aims to determine the quality of tap water in an industrial area, Hazaribagh (Human Rights Watch, 2012). Unsafe water is subjected to microbial contamination and as well as chemical impurities including heavy metals. It is important to evaluate the quality of water to prevent the transmission of pathogens by ingesting contaminated water. One of the most important global health challenges is to prevent waterborne diseases, which makes evaluation of water even more important (Craun G F, 2010) (Yoder JS, 2001-2002). One of the most profound pathogen that researchers are concerned about is Pseudomonas aeruginosa, which is likely to be found in tap water. Pseudomonas aeruginosa is rod-shaped aerobic bacteria consisting of a single polar flagellum (Novik G, 2015). It is an opportunistic pathogen known to lead to a wide spectrum of diseases that is eventually responsible for causing death (Kerr KG, 2009). It is responsible for causing nosocomial infections, hence, a threat to the hospitals around the industrial areas. One of the common infections includes ventilator-associated pneumonia (Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia.

2005). In addition to this, gastrointestinal tract infections (not endoscopy-related) and catheter-related bloodstream infections (Wendy IS, 2015) are also caused by P.aeruginosa. Individuals eligible to be infected by this pathogen are immunocompromised people and neonates (Jefferies JMC, 2012); (Kerr KG, 2009); (Leclerc H, 2002). The waste effluents released from industries may end up in the water sources along with the help of rain, flushing the contaminants from industrials waste dumping areas to the water sources. Health hazards caused by toxic levels of heavy metals can be responsible for central nervous function breakdown, lung, kidney and liver damage. Exposure to heavy metals for long term and accumulation of heavy metals in the body may result in the progression of muscular, physical, and neurological degenerative processes that mimic certain diseases such as Parkinson's disease and Alzheimer's disease (Monisha J, 2014). On a similar note, repeated long-term contact with some heavy metals may even damage nucleic acids, cause mutation, modify hormones thereby disrupting the endocrine and reproductive system and eventually lead to cancer (Jarup L, 2003). Besides *P.aeruginosa*, lead was found in a large amount in the water samples, except C3. Therefore, the result specify that *P.aeruginosa* can efficiently survive in the presence of lead. This feasibility was due to being resistant to the toxicity of lead. However, unlike *P.aeruginosa,* humans may not be entirely safe from water in these areas and longer exposure to lead will have consequences on health. On the other hand, chromium was not present in any of the sample examined. Therefore, the samples taken from the two areas are unconfined of chromium.

References

Belal AR, C. S. (2015). Corporate environmental responsibility and accountability: What chance in vulnerable Bangladesh? . *Critical Perspectives on Accounting*, 44-58.

Berthelot P, C. F. (2006). Magnetic valves as a source of faucet contamination with Pseudomonas aeruginosa? *Intensive Care Medicine*, 32(8), 1271-1271.

Beyenal H, C. S. (2003). The double substrate growth kinetics of Pseudomonas aeruginosa. *Enzyme and Microbial Technology*, 32, 92-98.

Bhandari, I., Colet, E., Parker, J., Pines, Z., Pratap, R., & Ramanujam, K. (1997, March). Advanced Scout: Data Mining and Knowledge Discovery in NBA Data. *Data Mining and Knowledge Discovery*, 121-125.

Bodey GP, B. R. (1983). Infections caused by Pseudomonas aeruginosa. *Clinical Infectious Diseases*, 5(2), 279-313.

Botzenhart K, &. D. (1993). Ecology and Epidemiology of Pseudomonas aeruginosa. In *Pseudomonas aeruginosa as an Opportunistic Pathogen* (pp. 1-18). Berlin, Germany: Springer.

Brian, S. (2012, January 25). The Problem of Shot Selection in Basketball. *PLoS One*. Retrieved from https://doi.org/10.1371/journal.pone.0030776

Brown V I & Lowbury J L. (1965). Use of an improved cetrimide agar medium and other culture methods for Pseudomonas aeruginosa. *Journal of clinical pathology*, 18 : 752.

Cedillo A, H. R. (2012). *Toxic Tanneries.* Dhaka: Human Rights Watch.

Choudhury, R. D., & Bhargava, P. (2007). Use of Artificial Neural Networks for Predicting the Outcome. *International Journal of Sports Science and Engineering, 1*(2), pp. 87-96.

DoE. (2014). *River Water Quality Report.* Dhaka: Ministry of Environment & Forest, Government of Bangladesh.

Duckworth, F., & Lewis, T. (1999). *Your Comprehensive Guide to the Duckworth/Lewis Method for Resetting Targets in One-day Cricket.* University of the West of England.

Duressa G, A. F. (2019). Assessment of Bacteriological and Physicochemical Quality of Drinking Water from Source to Household Tap Connection in Nekemte, Oromia, Ethiopia. *Journal of Environemntal and Public Health*, 7 pages.

Duruibe JO, O. M. (2007). Heavy metal pollution and human biotoxic effect. *International Journal of Physical Sciences*, 112-118.

Exner M, K. A. (2005). Prevention and control of health care–associated waterborne infections in healthcare facilities. *American Journal of Infection Control*, 33(5 Suppl), S26-S40.

Favero MS, C. L. (1971). Pseudomonas aeruginosa: Growth in Distilled Water from Hospitals. *Science*, 173, 836–838.

Fenwick A. (2006). Waterborne Infectious Diseases- Could they be Consigned to History? *Science*, 313, 1077- 1081.

Gadd G M & Griffiths A J. (1977-1978). Microorganisms and Heavy Metals Toxicity. *Microbial Ecology*, 4(4) : 303-317.

Garbarino J R, H. H. (1995). *Contaminants in the Mississipu River.* Virginia, USA: Geological Survey Circular 1133.

Gautam S P. (2013). *S.P. Gautam's research works.* Delhi: Central Pollution Control Board.

Google Image, U. L. (2013). *Bangladesh - Assistance with preparation for tannery relocation from Hazaribagh to Savar.* Savar: UNIDO: Leather Panel.

Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. (2005). *American Thoracic Society Documents*, 171:388–416.

Hardalo C, &. E. (1997). Pseudomonas aeruginosa: Assessment of risk from drinking water. *Critical Reviews on Microbiology*, 23(1), 47-75.

Haroun AA, K. K. (2017). Evaluation of Heavy Metal Tolerance Level (MIC) and Bioremediation Potentials of Pseudomonas aeruginosa isolated from Makera-kakuri Industrial Drain in Kaduna, Nijeria. *European Journal of Experimental Biology*, 7 (5): 28.

Hoque, M. A., Hoque, M. M., & Ahmed, K. M. (2007). Declining groundwater level and aquifer dewatering in Dhaka metropolitan area, Bangladesh: Causes and quantification. *Hydrogeology Journal*, 1523-1534.

Hutton M & Symon C. (1986). The quantities of cadmium, lead, mercury and arsenic entering the U.K. environment from human acitivities. *Science of The Total Environment*, 57, 129-150.

Islam M R, J. M. (2013). *Consumption of Unsafe Foods: Evidence from Heavy Metal, Mineral and Trace Element Contamination.* Dhaka, Bangladesh: National Food Policy Capacity Strengthening Programme.

Ivy N, H. M. (2015). Effects of Industrial Effluents on Germination and Early Growth of Selected Agricultural Crops. *Journal of Agronomy*, 14 (1): 43-48.

Jaishankar M, T. T. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*, 7(2): 60-72.

Jefferies JMC, C. T. (2012). Pseudomonas aeruginosa outbreaks in the neonatal intensive care unit- a systematic review of risk factors and environmental sources. *Journal of Medical Microbiology*, 61(Pt 8), 1052-1061.

Jolly Y N, I. A. (2013). Characterization of some industrial effluents of Dhaka city to asses their quality as irrigation water. *Bangladesh Journal of Physics*, 13, 1-7.

Keith P. (2005). Pseudomonas aeruginosa. In A. M. White D, *Frontiers in Antimicrobial Resistance.* (pp. 355-366). Washington DC: ASM Press.

Kerr KG, S. A. (2009). Pseudomonas aeruginosa: a formidable and ever present adversary. *Journal of Hospital Infection*, 73, 338-344.

King A & Phillips I. (1978). The identification of Pseudomonads and Related Bacteria in a clinical Laboratory. *Journal of Medical Microbiology*, 11(2): 165-176.

Kogure T. (2014). *The Leather World of Hazaribagh.* Hazaribagh, Dhaka: Research Initiative for Social Equity Society.

Leclerc H, S. L.-C. (2002). Microbial agents associated with waterborne diseases. *Critical Reviews in Microbiology*, 28, 371-409.

Leentech. (2004). *Water treatment and Air Purification* . Rotterdamsewag, Netherlands: Published by Lenntech.

Liu KJ, S. X. (2001). In vivo reduction of chromium (VI) and its related free radical generation. *Molecular and Cellular Biochemistry*, 222:41-47.

Majumder A. (2009). *Bangladesh River Pollution Threatens Millions.* . Retrieved from Reuters.

Mekonnen M M & Hoekstra A Y. (2016). Four billion people facing severe water scarcity. *Science Advances*, 2(2), e1500323-e1500323.

Mena KD & Gerba CP. (2009). Risk assessment of Pseudomonas aeruginosa in water. *Reviews of Environmental Contamination and Toxicology*, 71-115.

Mittal S K & Ratra R K. (2000). Toxic effect of metal ions on biochemical oxygen demand. *Water Research*, 34(1), 147-152.

NHS. (2015). UK Standards for Microbiology Investigations. *Bacteriology*, B 55(5.2): 1-21.

Novik G, S. V. (2015). An Insight Into Beneficial Pseudomonas bacteria. *Novik, G., Savich, V., & Kiselev, E. (2015). An Insight InMicrobiology in Agriculture and Human Health*, Chapter 5.

Petignat C, F. P. (2006). Exogenous sources of Pseudomonoas aeruginosa in intensive care unit patients: Implementation of Infection control measures and follow-up with molecular typing. *Infection Control and Hospital Epidemiology*, 27, 953-957.

Public Health England. (2015). *Identification of Pseudomonas species and other non- glucose fermenterrs.* UK: UK Standards for Microbiology Investigation.

Public Health Ontario. (2019). Public Health Inspector's Guide to Environmental Microbiology Laboratory Testing. *Public Health Ontario*.

Rahman Z & Singh V P. (2019). The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and Lead (Pb)) on the total environment: an overview. *Environmental Monitoring and Assessment.*, 191(7).

Rajkovic MB, L. C. (2008). Identification of Metals (Heavy and Radioactive) in drinking water by indirect analysis method based on Scale tests. *Sensors*, 8, 2188–2207.

Rasamiravaka T, L. Q. (2014). The formation of biofilms by Pseudomonas aeruginosa: A review of the natural and synthetic compounds interfering with control mechanisms. *Hindawi*.

Rogues AM, B. H. (2007). Contribution of tap water to patient colonisation with Pseudomonas aeruginosa in a medical intensive care unit. *Journal of Hospital Infection*, 67(1), 72-78.

Roy, P. (2019). *Tannery Relocations Impact: Situations getting better slowly.* Hazaribagh: The Daily Star.

Saha P K & Hossain M D. (2011). Assessment of Heavy Metal Contamination and Sediment Quality in the Buriganga River, Bangladesh. *2nd Internation Conference on Environmental Science and Technology. IPCBEE.* Singapore: IACSIT Press.

Sankaranarayanan, V. V., Sattar, J., & Lakshmanan, L. S. (2014). Auto-play: A data mining approach to ODI cricket simulation and prediction. *SIAM International Conference on Data Mining*, (p. 1064).

Schobert M, J. D. (2010). Anaerobic physiology of Pseudomonas aeruginosa in the cystic fibrosis lung. *International Journal of Medical Microbiology*, 300, 549-556.

Shikhlomanov L A. (1993). *World fresh water resources. In: Gleick, P.H. (Ed.), Water in Crisis:*. New York: Oxford University Press.

Siegrist J. (2007). *Pseudomonas Media and Tests.* Sigma-Aldrich.

Silbergeld EK, W. M. (2000). Lead as a carcinogen: Experimental evidence and mechanisms of action. . *American Journal of Industrial Medicine.*, 38(3):316-323.

Silva A A L, C. M. (2012). Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolation from sewage. *Brazilian Journal of Microbiology* , 1620-1631.

Strasser J. (2015). *Bangladesh's Leather Industry.* Bangladesh: Springer Geography.

The World Bank. (2018). *Enhancing opportunities for clean and resilient growth in urban Bangladesh.* 1818 H Street NW, Washington DC: The World Bank.

Tilzer M M, K. M. (1993). Hypertrophic and Polluted Freshwater Ecosystems: Ecological basis for water resource management. *Dept. of Botany, Dhaka University*.

Trautmann M, L. P. (2005). Ecology of Pseudomonas aeruginosa in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. *American Journal of Infection Control*, 33 (5 Suppl), S41-S49.

Tulabandhula, T., & Rudin, C. (2014). Tire Changes, Fresh Air, and Yellow Flags: Challenges in Predictive Analytics for Professional Racing. *Big data*. Retrieved from http://doi.org/10.1089/big.2014.

van der Kooij D, O. J. (1982). Growth of Pseudomonas aeruginosa in tap water in relation to utilization of substrates at concentrations of a few micrograms per liter. *Applied and Environmental Microbiology*, 44, 986-1095.

Verma R & Dwivedi P. (2013). Heavy metal water pollution- A case study. *Recent Research in Science and Technology*, 5(5): 98-99.

Warburton D, B. B. (1994). The survival and recovery of Pseudomonas aeruginosa and its effect upon salmonellae in water:. *Canadian Journal of Microbiology*, 40, 987-992.

Wendy IS, T. D. (2015). Nosocomial Gram-negative bacteremia in intensive care: epidemiology, antimicrobial susceptibilities, and outcomes. *International Journal of Infectious Diseases*, 37, 129-134.

WHO. (1997). *Surveillance and control of community supplies. Guidelines for drinking-water quality.* Geneva: WHO Library Cataloguing in Publication Data.

WHO. (2003). *Chromium in Drinking-water.* Geneva, Switzerland: WHO publications.

WHO. (2017). *Guidelines for Drinking-water quality: fourth edition incorporating the first addendum.* Geneva: WHO Library Cataloguing-in-Publication Data.

WHO. (2018). *Sustainable Development and Healthy Environment.* Retrieved from WHO Bangladesh.

Williams MM, A. C. (2013). Plumbing of hospital premises is a reservoir for opportunistically pathogenic microorganisms: A review. *Biofouling: The Journal of Bioadhesion and Biofilm Research,* 29, 147-162.

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	Amounts (g/L)
Nutrient agar	28

2. **Cetrimide Agar**

Ingredients	Amounts (g/L)
Cetrimide agar	45.3

3. **Trypticase soy broth, (Oxoid, England)**

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
Final pH	7.3±0.2

4. **Dextrose fermentation**

Ingredients	Amount (g/L)
Peptone	10
Beef extract	1
Dextrose	5
Sodium chloride	5.0
Phenol red	0.0018

5. Sucrose fermentation

Ingredients	Amount (g/L)
Peptone	10
Beef extract	1
Sucrose	5
Sodium chloride	5.0
Phenol red	0.0018

6. Lactose fermentation

Ingredients	Amount (g/L)
Peptone	10
Beef extract	1
Lactose	5
Sodium chloride	5.0
Phenol red	0.0018

7. Mannitol fermentation

Ingredients	Amount (g/L)
Peptone	10
Beef extract	1
Mannitol	5
Sodium chloride	5.0
Phenol red	0.0018

8. Gelatine hydrolysis

Ingredients	Amount (g/L)
Peptone	5
Beef extract	3
Gelatine	120

9. Simmon's citrate agar (Difco, USA)

Ingredients	Amount (g/L)
Simmon Citrate agar	24.28

10. MIU test

Ingredients	Amount (g/L)
MIU base medium	95
Urea	40

11. Nitrate Reduction

Ingredients	Amount (g/L)
Peptone	5
Beef extract	3
Nitrate	1
Sodium chloride	13

12. T_1N_1 soft agar

Ingredients	Amount (g/L)
Tryptone	10 g
Sodium chloride	10 g
Agar	6 g

APPENDIX II

1. Kovac's reagent

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

1. Barritt's 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^oC.

Solution B

10 g of 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^oC.

2. 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.

3. Catalase reagent

18ml distilled water was dissolved with 2ml hydrogen peroxide and stored in light protective bottle

4. Grams Staining reagents

- Gram's Crystal Violet (Solution A)
  -Crystal Violet – 2.000gm
  - Ethyl alcohol – 20.000ml (95%)

- Gram's Crystal Violet (Solution B)
  - Ammonium oxalate – 0.800gm
  - Distilled Water – 80.000ml

Solution A and B are mixed and stored for 24 hours before use. The resulting stain is stable

- Gram's Decolourizer
  - Ethyl alcohol – 50.0ml (95%)
  - Acetone – 50.0ml

- Gram's Iodine
  - Iodine – 1.00gm
  - Potassium iodide – 2.00gm
  - Distilled water – 300.00ml

- Safranin (0.5%)
  - Safranin o – 0.500gm
  - Ethyl alcohol – 100.00ml (95%)

APPENDIX III

Instruments

The important equipment used through the study are listed below:

- Autoclave, Model no: HL-42AE : Hirayama corp, 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
- Atomic Absorption Spectrometer : Shimadzu, AA7000 series