Analysis of physicochemical parameters and microbial status in Gulshan lake water

Analysis of physicochemical parameters and microbial status in Gulshan lake water

Submitted by

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8th September , 2014.

DECLARATION

I hereby declare that this work **Analysis of physicochemical parameters and microbial status in Gulshan lake water** is my own and that, to the best of my knowledge and belief, it contains neither materials previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institutes, except where due acknowledgment has been made in the text.

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ABBREVIATIONS

°C	:	Degree Celsius
mg	:	Milligram
gm	:	Gram
L	:	Liter
ml	:	Milliliter
p1	:	Microliter
e.g.	:	For example
et al.	:	And others
pH	:	Negative logarithm of hydrogen ion concentration
%	:	Percentage
spp.	:	Species

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ABSTRACT

Microbiological evaluation and physicochemical parameters of lake comprises detection of indicator bacteria of water pollution and pathogenic bacteria present in the surface layers of water. Polluted water has a significant impact on human health. Therefore examination of lake water for the presence of bacteria indicatory of sanitary state and of pathogenic bacteria as well as physicochemical parametes may supply information on water quality of the lake. In the present study, water samples were collected from three different sites of Gulshan lake of Dhaka city based on human activities from February 2014 to May 2014(summer to early rainy season). Different physicochemical parameters (pH, DO, temperature, TDS, conductivity) were measured and were inoculated into five different selective agar media, MacConkey, Mannitol salt agar (MSA), thiosulphate-citrate-bile salt-sucrose (TCBS), cetrimide agar and m-FC Agar to detect the presence of pathogenic bacteria. The overall bacterial count was high particularly on site3. Cultural and biochemical examinations revealed the presence of *Escherichia coli, Enterobacter, Staphylococcus aureus, Pseudomonas* and *Vibrio species (V. cholerae, V. parahaemolyticus, V. mimicus and V alginolyticus*) in the water samples. The results indicate that Gulshan lake water serves as a reservoir of various pathogenic bacteria and that people using the water might get diseases. This study emphasizes the need for elaborated microbiological examinations of water from Gulshan and other freshwater lakes.

1. Introduction

1.1 Background

Water is one of the easiest vehicles for some of the pathogenic organisms and the contaminating water bodies may help in the out break of epidemic diseases The pathogenic most frequently transmitted though water are those which cause infection of the intestinal tract, namely typhoid, paratyphoid diarrhea, dysentery and cholera (Pelezar and Reid, 1978). Water borne diseases constitute a major health burden in Bangladesh. According to Bangladesh health and injury report on children under 5 in 2005, children die every year from diarrhea (Bangladesh Health and Injury Survey Report, 2005). In the. humid, tropical region of Bangladesh, outbreaks of diarrheal diseases, often on an epidemic scale, are not unusual and the possible role of water-borne pathogens in these outbreaks has been emphasized (Khan et al., 1992).

Water can contain and support very large and genetically diverse bacterial populations that may include pathogenic strains. The release of bacteria with resuspended sediments can create elevated concentrations of bacteria in water far above regulatory thresholds. Current microbial water quality models, that are used to support management decisions, ignore this bacterial input by sediment. It has been known for some time that substantial populations of fecal-coliforms and *E coil* are harbored in freshwater both in surface and grown. However, the relative importance of sediments as bacterial habitats and as a source of water-borne fecal-coliforrns and *E. coil* has not been recognized until recently when a large number of publications have shown that in many cases the resuspension of sediment, rather then runoff from surrounding lands, can create elevated *E. coli* concentrations in water. The survival of enteric bacteria in aquatic ecosystems has received considerable attention because of the dangers that pathogenic members of the group pose to humans. The present study was conducted to identify fecal coliforms and pathogenic bacteria e.g. *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Pseudomonas and Vibrio species (V. cholerae*, *V. parahaemolyticus*, *V. mimicus* and *V.alginolyticus*) from water and sediment of Dhanmondi Lake in Dhaka city. The detection of pathogenic bacteria in water will help in controlling water borne infection in this region.

1.2 Literature review

1.2.1 Lake ecosystem

Lakes are either natural or artificial wetland of usually standing or slow-moving water body. For shallow water bodies rooted plants can grow over most of the bottoms. Energy source of a lake ecosystem is the sun light. Within the lake ecosystem different communities of biological species are distributed throughout the depth. Relatively small portion of water is in contact with the air and decomposition takes place on the bottom. Variation of oxygen level with depth, light, temperature, water quality such as turbidity, hardness, mineral content, acidity etc., profoundly influence life in the lake, its distribution, and adaptation. The lake's open water is dominated by minute suspended organisms called phytoplankton (Pinaki, 2008).

1.2.2 Sources of pollution and its effects on lake environment

The general objective for all water management is to make sure that all waters are free from contaminating levels of substances and materials mainly as a result of direct or indirect human activities of various types. Environmental deterioration of the lake can be caused by many different reasons. For example, when sewage is discharged into a lake or other water body, it changes biological activities in the lake water by changing turbidity, temperature, soluble dissolved oxygen content, nutrient like nitrogen, phosphorus, and other minerals. Lack of dissolved oxygen causes anaerobic decomposition especially at the bottom layer accompanied by the production of gases like ammonia and hydrogen sulfide. Dc-oxygenation kills fish. In addition to sewage discharges, urban surface run-off and storm water discharges carry toxic materials, suspended particles which ultimately settle at the bottom of the lake. Over the years, as the concentration of these chemicals start to build up and exceed certain limits, the water quality of the lake begins to deteriorate -- so is the intended use of the lake or the water body. One such phenomenon is known as eutrophication or offensive, bloom of plants in the lake water which is associated with enrichment of lake water by phosphate. This gives rise to sudden bloom of aquatic plants that ultimately cover the lake surface blocking penetration of sunlight to the layer below and thereby hamper photosynthesis. The algae die and settle at the bottom. Accumulation of the organic materials creates depletion of oxygen at the lake bottom. This results in destruction of aquatic lives of the lake (Hossain, 2010).

1.2.3 Microbial population of lake

A number of bacteria occur naturally in lake water streams. Some are found living in the water and sediments as photosynthetic autotrophs or a saprophytes living on dead matter. Others exist in or on other organisms as mutual symbiotic, commensals. Certain bacteria that live in the intestinal tracts of animals are essential for the recovery of nutrients from digested food. Millions of these naturally occurring organisms are passed out of the body with fecal wastes. If pathogenic (disease-causing) organisms are present, they may be passed as well. When a stream is polluted by fecal material, pathogenic bacteria may be introduced, posing a health hazard to those who come in contact with that water such as cholera (*Vibrio cholerae*), typhoid fever (*Salmonella typh*i), and gastroenteritis (*Escherichia coli*) (Waterbome Pathogen, 2011).

1.2.4 Pathogens of fresh water environments

Some of the major bacterial pathogens spread via contact with contaminated water. The sources of contamination are most often animal or human focal wastes or infected humans and other animals. Fresh water can serve both as reservoir for the pathogenic bacteria and as a vehicle of transmission of disease (Benenson, 1995). Lake water is also a natural reservoir of common bacteria, water-borne human pathogens such as *Shigella* species. *Salmonella* species, *E. coli* 0157:H7, enterotoxigenic*E. coli*, *Vibrio cholerae* 01, and *Vibrio parahaemolyticus* (APHA, 1992) and opportunistic pathogens such as *Aeromonashydrophila*and *Pseudomonasaeruginosa* (Auerbach et al., 1987; Graevenitz, 1985).

Escherichia coli

Escherichia coli is present in large numbers in the normal intestinal flora of humans and animals, where it generally causes no harm. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. A limited number of enteropathogenic strains can cause acute diarrhoea. Several classes of enteropathogemc*E. coli* have been identified on the basis of different virulence factors, including enterohaemorrhagic*E. coli* (EHEC), enterotoxigenic*E. coli* (ETEC), enteropathogenic*E. coli* (EPEC), enteroinvasive*E. coli* **(EIEC)**

EHEC serotypes, such as *E. coli* 0157:H7 and *E. coli* 0111, cause diarrhoea that ranges from mild and non-bloody to highly bloody, which is indistinguishable from haemorrhagic colitis. ETEC produces heat-labile or heat-stable enterotoxin, or both toxins simultaneously, and is an important cause of diarrhoea in developing countries, especially in young children. Symptoms of ETEC infection include mild watery diarrhoea, abdominal cramps, nausea and headache. Infection with EPEC has been associated with severe, chronic, non-bloody diarrhoea, vomiting and fever in infants. EIEC causes watery and occasionally bloody diarrhoea where strains invade colon cells by a pathogenic mechanism similar to that of *Shigella* (Nataro and Kaper, 1998). Humans are the major reservoir of EPEC, ETEC and EIEC strains. On the other hand, livestock, such as cattle, sheep, goats, pigs and chicken are a major source of EHEC strains.

Infection is associated with person-to-person transmission, contact with animals, food and consumption of contaminated water. Person-to-person transmissions are particularly prevalent in communities where there is close contact between individuals, such as nursing homes and day care centers (O'Connor et al., 2002).

Recently, a fifth category of *E. coli*, recognizable by its aggregative or "stackedbrick" type of adherence to cultured mammalian cells, has been recognized as yet another category of diarrheagenic*E. coli* in children in different parts of the world. Because of its characteristic aggregative type of adherence, this *E. coli* has been referred to as enteroaggregative*E. coli* (EAggEC). The diagnosis of EAggEC is dependent upon cell culture adherence assays, both of which are expensive and cumbersome and are beyond the reach of the majority of clinical laboratories. Recently a research in Thailand showed that EAggEC forms bacterial clump which is visible as a thick scum in a liquid culture e.g. Mueller-Hinton broth, nutrient broth or Luria broth (Nataro and Kaper, 1998).

Vibrio spp.

The genus Vibrio is in the family *Vibrionaceae*. Members of the *Vibrio* genus are straight or curved Gram-negative, nonspore-forming rods. *V. vulnificus* is similar phenotypically to *V.parahaemolyticus*. All *vibrios* are ubiquitous in the marine environment. There are 30 species in the genus *Vibrio*; thirteen of these are pathogenic to humans, including *V. cholerae*, *V.mimicus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus*, All of the pathogenic. *vibrios*

have been reported to cause foodborne and waterborne diseases, although *V cholerae*01, *V. parahaemolyticus*, and V. vulnificus are considered the most significant agents. (Atlas, 1997).

Staphylococcus spp.

The *Staphylococcus* genus includes at least 40 species. Of these, nine have two subspecies and one has three subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Members of the *Staphylococcus* frequently colonize the skin and upper respiratory tracts of mammals and birds. Some species specificity has been observed in host range, such that the *Staphylococcus* species observed on some animals appear more rarely on more distantly related host species. *Staphylococcus* can cause a wide variety of diseases in humans and animals through either toxin production or penetration. *Staphylococcal* toxins are a common cause of food poisoning, as they can be produced by bacteria growing in improperly stored food items. The most common sialadenitis is caused by *staphylococci*, as bacterial infections.

Pseudomonas spp.

Pseudomonas is a genus of Gram-negative, aerobicgammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonise a wide range of niches.Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genomesequences has made the genus an excellent focus for scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. Fluorescens*. Infectious species include *P. aeruginosa*, *P. oryzihabitans*, and *P. plecoglossicida*. *P. aeruginosa* flourishes in hospital environments, and is a particular problem in this environment, since it is the second-most common infection in hospitalized patients (nosocomial infections)[*citation needed*]. This pathogenesis may in part be due to the proteins secreted by *P. aeruginosa*. The bacterium possesses a wide range of secretion systems, which export numerous proteins relevant to the pathogenesis of clinical strains.

Fecal coliform

A fecal coliform is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulatingbacterium. Coliform bacteria generally originate in the intestines of warm-blooded animals. Fecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactose within 48 hours at 44 ± 0.5°C. The term "thermotolerant coliform" is more correct and is gaining acceptance over "faecal coliform". Coliform bacteria include genera that originate in feces (e.g. *Escherichia*) as well as genera not of fecal origin (e.g. *Enterobacter*, *Klebsiella*, *Citrobacter*). The assay is intended to be an indicator of fecal contamination; more specifically of *E. coli* which is an indicator microorganism for other pathogens that may be present in feces. Presence of fecal coliforms in water may not be directly harmful, and does not necessarily indicate the presence of feces. The presence of fecal coliform in aquatic environments may indicate that the water has been contaminated with the fecal material of humans or other animals. Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural and storm runoff, and from human sewage. However, their presence may also be the result of plant material, and pulp or paper milleffluent. Pets, especially dogs, can contribute to fecal contamination of surface waters. Runoff from roads, parking lots, and yards can carry animal wastes to streams through storm sewers. Birds can be a significant source of fecal coliform bacteria. Swans, geese, seagulls, and other waterfowl can all elevate bacterial counts, especially in wetlands, lakes, ponds, and rivers. Large quantities of fecal coliform bacteria in water are not harmful according to some authorities, but may indicate a higher risk of pathogens being present in the water. Some waterborne pathogenic diseases that may coincide with fecal coliform contamination include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A. The presence of fecal coliform tends to affect humans more than it does aquatic creatures, though not exclusively. Untreated organic matter that contains fecal coliform can be harmful to the environment. Aerobic decomposition of this material can reduce dissolved oxygen levels if discharged into rivers or waterways. This may reduce the oxygen level enough to kill fish and other aquatic life. Reduction of fecal coliform in wastewater may require the use of chlorine and other disinfectant chemicals. Such materials may

kill the fecal coliform and disease bacteria. They also kill bacteria essential to the proper balance of the aquatic environment, endangering the survival of species dependent on those bacteria. So higher levels of fecal coliform require higher levels of chlorine, threatening those aquatic organisms.

1.3 Water Quality Parameters

Contamination of water-bodies is a major concern in today's era. The biological wealth of a water body is mainly dependent on its water quality and it is of major issue of concern to mankind toady. Decrease in water quality (unfit for human consumption) is also attributed to the fact that today most water bodies are been loaded with toxic material and chemicals, human and industrial waste, organic matter, and religious rituals of Idol immersions.Water samples were collected from three sites and were analyzed for various water quality parameters such as pH, total dissolve solids (TDS), conductivity, dissolved oxygen (OD), Biological Oxygen Demand (BOD), and following standard methods.

1.4 Aims and objectives of the present study

Gulshan Lake is the northernmost lake in a chain of water bodies (Gulshan Lake, Hatirjheel, BegunbariKhal, Balu River and Shitalakhya River) in Dhaka, suffering from highly significant pollution. Gulshan Lake with an area of about 100 ha and is located at 23o48' N and 90o25' E of Dhaka city. The length of the lake is 3.8 km which covers an area of 0.0160 km2. It has an average depth of 2.5 m and a volume of 12 ×105 m3. The peripheral sides are, northern at Baridhara, southern at Tejgaon-Hatirjheel, western at Gulsan-Banani and eastern at Badda area. Gulshan Lake is one of the major of few remaining water bodies of Dhaka city; not only is its presence important for the sustenance of the eco-system, it is also considered as major main source of groundwater recharge at those area. Gulshan Lake is the northernmost lake of the chain of water bodies in Dhaka (Gulshan Lake, Hatirjheel, BegunbariKhal, Balu River and Shitalakhya River) increasing pollution from north to south. Due to proper supervision of the regulatory and maintenance body, Gulshan-Baridhara Lake was declared an Ecologically Critical Area (ECA) in 2001.The water of the lake is polluted day by day and during the dry season the level goes down and the pollution

becomes worst. Lack of proper maintenance of existing drainage system results indiscriminate drainage outlets which dispose untreated domestic and commercial wastewater and dumping of solid wastes and moreover encroachment has degraded the overall quality of the Lake. Sewage from the Badda, Baridhara, GulshanandBanani residential areas along with toxic discharges from the nearby industries have contaminated the water of Gulshan Lake also. The poor water quality of Gulshan Lake is also contributing to the pollution of Hatirjheel, where a restoration project is already implemented and the downstream water bodies. It is very important to take immediate steps to restore the water quality of Gulshan Lake.

Therefore, the specific objectives of the current study were:

I. Isolation of pathogenic bacteria from water samples collected from different sites of Gulshanlake using selective microbiological media.

2. Identification of the isolated bacteria using cultural and biochemical characteristics.

3. Measuring different parameters of water quality.

2. Materials and Methods

2.1 Collection of Samples

Sediment samples were collected from three (3) sites of the Gulshan lake from February 2014 to May 2014. The samples were aseptically collected from water surface using sterile containers. The sampling containers were marked properly and transported to the laboratory as early as possible. Samples were labeled in the field and transported to the laboratory and were processed in the Laboratory of Microbiology, BRAC University within 3 hours of collection. Then different physicochemical parameters (PH, DO, temperature, TDS, conductivity) were measured by using PH meter, dissolved oxygen (DO) meter, thermometer, conductivity meter and TDS meter.

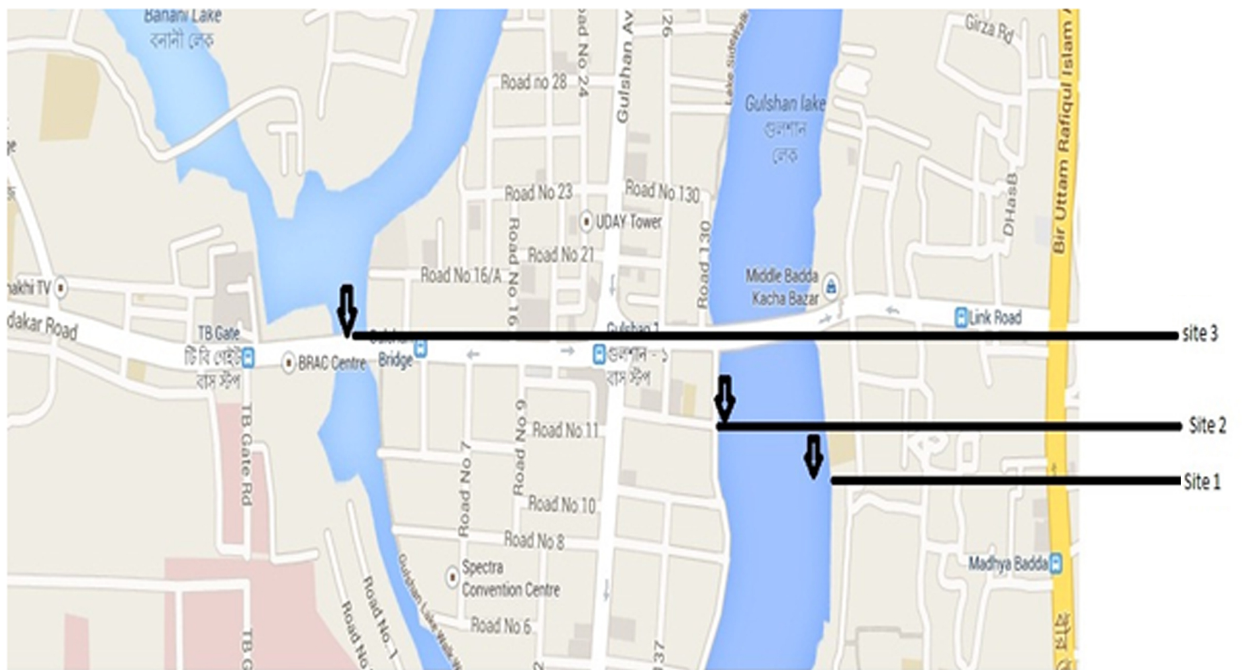

Figure 2.1: Map of Gulshan lake showing the sampling sites. Site A: boat station Korile,

Site B; boat station Gulshan1, Site C: boat station Badda.

2.2 Isolation of bacteria water sample

Five different selective agar media, MacConkey, Mannitol salt agar (MSA), thiosulphate-citrate-bile salt-sucrose (TCBS), Cetrimide agar and m-FC agar media were used for isolation of *Escherichia coli, Enterobacter, Staphylococcus aureus, Pseudomonas* and Vibrio species respectively. Various dilutions of the samples were spread on different selective media . All the plates were then incubated at 37 °C for 20 to 36 hours. M-Fc plate was incubated in 45°C. After incubation, every plate was observed carefully. Pink or red and colorless colonies were observed on MacConkey agar media, yellow of green colonies were observed on TCBS agar media and yellow, red and red with black centered colonies were observed on MSA agar media. According to 'Microbiological Laboratory Manual' by Cappuccinos and Sherman (1999), colony morphology of various isolates were examined and recorded on the basis of size, form, pigmentation, margin ,elevation and opacity.

2.3 Microscopic observation of isolates

For evaluation of microscopic character, pure colony of each isolates was picked and Gram staining was performed according to Hacker's modified method (Doetsch, 1981). The size, shape, arrangement and Gram reaction properties of isolates were carefully observed .

2.4 Biochemical characteristics of the isolates

According to 'Microbiological Laboratory Manual' by Cappuccion and Sherman (1999), following biochemical tests were performed for the identification of bacteria.

Catalase test

Catalase is an enzyme that splits H_2O_2 into water and O_2. This test is performed to differentiate between groups of microorganism on the basis of catalase production. 3% H_2O solution was added to each of the slides and a portion of the bacterial colony was mixed with it. Production of bubble indicates presence of catalase enzyme in the bacteria.

Oxidase test

Oxidase test was performed to differentiate between enteric and non-enteric bacteria. A portion of the colony was picked up with a tooth pick and rubbed on a strip of a

filter paper impregnated with oxidase reagent (1% aqueous solution of N’N’N’N’12 tetraniethyl-p-phenylenediamine dihyrochioride). Positive test is indicated by the presence of dark purple color within 10 seconds.

Triple Sugar Iron (TSI) agar test

This test was performed to assess the mode of sugar utilization by stabbing the butt and streaking the bacteria over the slant of Triple Sugar Iron (TSI) agar media. Formation of acid from sugar in fermentative mode is indicated by yellowing of the butt and slant. If gas was formed during the fermentation, it was show in the butt either by the formation of bubbles or cracking of the agar.

Motility Indole Urea (MIU) test

The test was carried out in motility indole urea semisolid medium. One suspected isolated colony is touched with a straight wire and was stabbed into carefully down the tubes without touching the bottom. Following incubation, the tubes were observed for the presence of motile organisms which disperse through the medium leaving the stab line and made the tube turbid. Production of cherry red reagent layer after addition of Kovac’s reagent in MIU medium demonstrates that the substrate tryptophan has been was hydrolyzed which d indicates indole positive reaction.

Citrate Utilization test

Citrate utilization by the isolates was observed by the growth of on slants of Simmons citrate agar. Following incubation, citrate positive culture was identified by the presence of growth on the surface of the slant and deep prussian blue coloration of the medium. Citrate negative was identified by no growth and the green coloration of the medium.

Methyl Red (MR) and Voges-Proskaur (VP) tests

MR-VP broth was inoculated and incubated at 37 °C for 48 hours. After incubation, the broth culture was divided into two portions. In one portion, methyl red reagent was added. A distinct red color and yellow color indicate positive and negative reaction respectively. In the other portion
of the broth, Barritt’s reagent A (6% a-naphthol) and B (40% KOH) were added and formation of pink color indicates positive reaction.

Carbohydrate Utilization

When carbohydrates are fermented by bacteria, they produce acidic products. A change in pH can be detected when fermentation of a given carbohydrate has

occurred. Acids lower the pH of the medium, which turns the media to Yellowcolor . When bacteria does not ferment the carbohydrate, the media remains red. Sometimes during fermentation, gas is produced. The Durham tube will then have a gas bubble trapped within it. There are three carbohydrate tests that can be performed. They are the Glucose (Dextrose) test, Lactose Test and the Sucrose Test. In all of these tests, the bacteria will be inoculated to the medium using a transfer loop. The results obtained will be similar to that illustrated in the picture below. The left tube shows less acid formation than the far right tube, but gas is still produced in this case. The Centre tube shows no carbohydrate utilization as there is neither any acid or gas produced. The medium remains the same. The Right tube shows acid is produced as evidenced by the yellow colouration; and gas is produced (an air bubble is trapped in the Durham tube).

Maintenance and preservation of isolates

The isolation and purification of bacterial isolates from different water samples were investigated. Typical and atypical colonies were picked up and streaked on nutrient agar plate. After 24 hours of incubation at 37°C, all the isolates were inoculated in vials containing nutrient agar slant with sterile paraffin and preserved at 4°C.

3. Results

In the present study, water samples were collected from three different sites of Gulshan lake of Dhaka city based on human activities from February 2014 to May 2014(summer to early rainy season). Different physicochemical parameters (PH, DO, temperature, TDS, conductivity) were measured and were inoculated into five different selective agar media, MacConkey, Mannitol salt agar (MSA), thiosulphate-citrate-bile salt-sucrose (TCBS), Cetrimide agar and m-FC Agar to detect the presence of pathogenic bacteria.

The result section can be divided into three parts:

- Bacterial load analysis of water samples collected from three sites.
- Physiochemical quality analysis of collected samples.
- Cultural and biochemical examinations of isolates.

The three different sites were:

Site A: boat station Korile,

Site B; boat station Gulshan1,

Site C boat station Badda.

Figure 3.1: Sample collecting site (Korile boat station)

Date of collection: 15th February

Date of spreading: 15th February

Date of reading: 16th February

Sample: Water

Collection place:Gulshan lake

Incubation time: 24 hours

Table 3.1: Microbial load in different selective media in February

Site	Membrane Faecal Coliform Agar (m_FC) (CFU/ml)	MacCoonkey (CFU/ml)	Cetrimide agar (CFU/ml)	Mannitol salt agar (MSA),(CFU/ml)	thiosulphate-citrate-bile salt-sucrose (TCBS), (CFU/ml)
1	7.2×10^{3}	1.19×10^{4}	5.00×10	8.6×10^{3}	7.1×10^{3}
2	3.9×10^{3}	1.58×10^{4}	3.00×10	4.3×10^{3}	4.4×10^{3}
3	1.7×10^{3}	1.76×10^{4}	8.00×10	5.3×10^{3}	1.34×10^{4}

Table 3.2: Water Quality Parameters

Site	Conductivity (µS)	Dissolved Oxygen mg/L	Total Dissolved Solids (TDS)	pH	Temperature (°C)
1	0.71	5.0	0.04	7.8	26
2	0.66	5.2	0.03	8.0	26
3	0.64	4.9	0.03	8.0	27.5

Date of collection: 15th March

Date of spreading: 15^{th}March

Date of reading: 16th

MarchSample: Water

Collection place: Gulshan lake

Incubation time: 24 hours

Table 3.3: Microbial load in different selective media in March.

Site	Membrane Faecal Coliform Agar (m_FC) (CFU/ml)	MacCoonkey (CFU/ml)	Cetrimide agar (CFU/ml)	Mannitol salt agar (MSA),(CFU/ml)	thiosulphate-citrate-bile salt-sucrose (TCBS), (CFU/ml)
1	2.31×10^4	1.9×10^3	4.00×10	8.6×10^3	6.3×10^3
2	1.31×10^4	7.0×10^3	2.00×10	4.3×10^3	1.9×10^3
3	1.66×10^4	1.3×10^3	9.00×10	5.3×10^3	2.51×10^4

Table 3.4: Water Quality Parameters

Site	Conductivity (µS)	Dissolved Oxygen mg/L	Total Dissolved Solids (TDS)	pH	Temperature (°C)
1	0.69	5.2	0.04	7.6	26
2	0.68	5.1	0.03	7.9	26.5
3	0.75	4.8	0.05	8.1	27.5

Date of collection: 15th April

Date of spreading: 15th April

Date of reading: 16thApril

March Sample: Water

Collection place: Gulshan lake

Incubation time: 24 hours

Table 3.5: Microbial load in different selective media in April.

Site	Membrane Faecal Coliform Agar (m_FC) (CFU/ml)	MacCoonkey (CFU/ml)	Cetrimide agar (CFU/ml)	Mannitol salt agar (MSA),(CFU/ml)	thiosulphate-citrate-bile salt-sucrose (TCBS), (CFU/ml)
1	4.0×10	1.14×10^4	1.00×10	1.64×10^4	3.6×10^3
2	9.0×10	1.58×10^4	1.00×10	1.29×10^4	1.9×10^3
3	2.17×10^4	1.76×10^3	11.00×10	2.8×10^3	3.9×10^3

Table 3.6: Water Quality Parameters

Site	Conductivity (µS)	Dissolved Oxygen mg/L	Total Dissolved Solids (TDS)	pH	Temperature (°C)
1	0.61	4.5	0.03	7.9	28
2	0.64	4.4	0.03	7.8	28
3	0.69	3.5	0.05	8.0	29

Date of collection: 15th May

Date of spreading: 15th May

Date of reading: 16thMay

March Sample: Water

Collection place: Gulshan lake

Incubation time: 24 hours

Table 3.7: Microbial load in different selective media in May.

Site	Membrane Faecal Coliform Agar (m_FC) (CFU/ml)	MacCoonkey (CFU/ml)	Cetrimide agar (CFU/ml)	Mannitol salt agar (MSA),(CFU/ml)	thiosulphate-citrate-bile salt-sucrose (TCBS), (CFU/ml)
1	1.1×10^2	1.3×10^3	1.00×10	1.5×10^4	1.8×10^3
2	9.0×10^2	1.8×10^3	1.00×10	1.2×10^4	1.3×10^3
3	1.7×10^3	1.63×10^4	9.00×10	2.8×10^3	2.3×10^3

Table 3.8: Water Quality Parameters

Site	Conductivity (µS)	Dissolved Oxygen mg/L	Total Dissolved Solids (TDS)	pH	Temperature (°C)
1	0.69	4.9	0.04	7.7	29
2	0.65	4.7	0.04	7.9	29
3	0.63	3.8	0.05	8.1	29.5

While comparing the overall result it was found that the bacterial count on MacConkey agar ranged between 1.3×10^{3}cfu/ ml and 1.76×10^{4} cfu/ml, maximum count was observed in the sample of site1 during summer. The highest count of bacteria (2.17×10^{4} cfu/100 ml) on m-FC agar was found in site 3 during rainy season, while the lowest count (4) was found in site1. Bacterial count on cetrimide agar ranged between 0 and10cfu/ml during the whole time period. In TCBS agar the overall count was high. In MSA agar the count increased during the rainy season in all three sites .The physical properties of the water samples are given in above tables. In summer, the water temperature ranged between 25 and 27°C, while in Rainy season that was around 29°C. The results indicate a favorable temperature for bacterial growth throughout the time period. The maximum pH (8.1) was detected in the site 3, while the minimum pH (7.6) was seen in the sample of site1.According to the United State Public Health (USPH), drinking water standards are pH 6.0 to 8.5DO (dissolved oxygen) was in a range between 3.5 to 5.5 mg/l.

Table 3.9 (a): Colony characteristics of the isolates on MacConkey agar media

Collection Site	Isolate No	Colony characteristics				
		Size	Form	Color	Margin	Elevation
Site 1	1	Large	Circular	Pink	Entire	Raised
	2	Large	Circular	Dark pink	Entire	Raised
	3	Small	Circular	Colorless	Entire	Raised
Site 2	4	Large	Circular	Pink	Entire	Raised
	5	Medium	Circular	Colorless	Entire	Convex
	6	Small	Circular	Pink	Entire	Raised
Site 3	7	Large	Circular	Colorless	Entire	Raised
	8	Small	Circular	Pink	Entire	Convex
	9	Pinpoint	Circular	Colorless	Entire	Raised

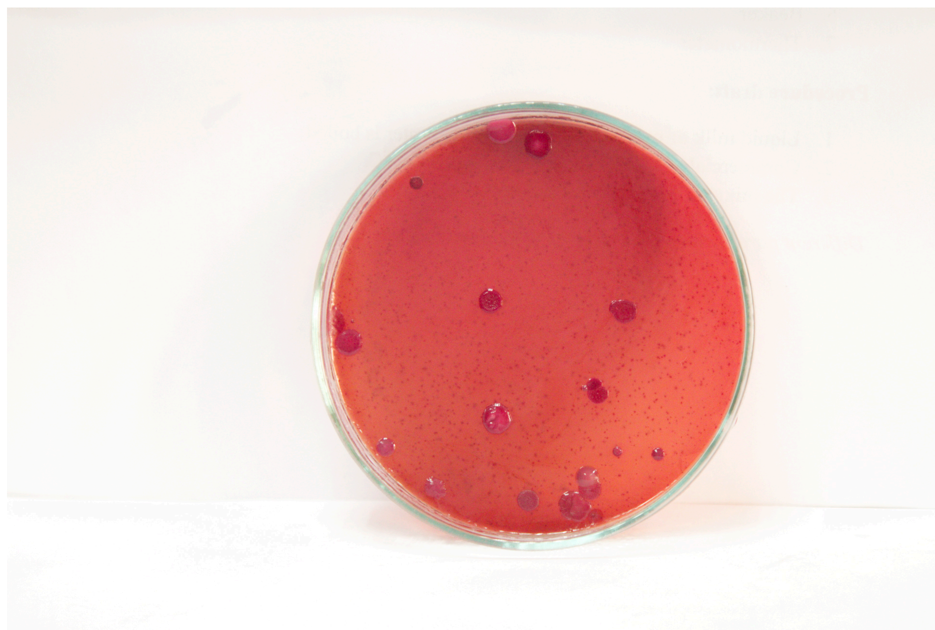

Figure 3.2 (a): Bacterial colonies on MacConkey agar media. Pink colonies and colorless colonies indicate lactose fermenter and non-fermenter respectively.

Table 3.9 (b): Colony characteristics of the isolates on TCBS agar media.

Collection Site	Isolate No	Colony characteristics				
		Size	Form	Color	Margin	Elevation
Site 1	10	Small	Circular	Green	Entire	Convex
	11	Small	Irregular	Yellow	Undulate	Convex
Site 2	12	Small	Circular	Green	Entire	Raised
	13	Medium	Circular	Yellow	Entire	Convex
Site 3	14	Large	Circular	Yellow	Entire	Convex
	15	Medium	Circular	Green	Entire	Convex

Figure 3.2 (b): Bacterial colonies on TCBS agar media. Green colonies and yellow colonies indicate lactose fermenter and non-fermenter respectively.

Table 3.9 (c): Colony characteristics of the isolates on m-FC agar media

Collection Site	Isolate No	Colony characteristics				
		Size	Form	Color	Margin	Elevation
Site 1	16	Small	Circular	Blue	Entire	Convex
	17	Medium	Irregular	Light-blue	Undulate	Convex
Site 2	18	Medium	Circular	Colorless	Entire	Raised
	19	Large	Circular	Blue	Entire	Raised
Site 3	20	Large	Circular	Light-blue	Entire	Convex
	21	Medium	Circular	Blue	Entire	Raised

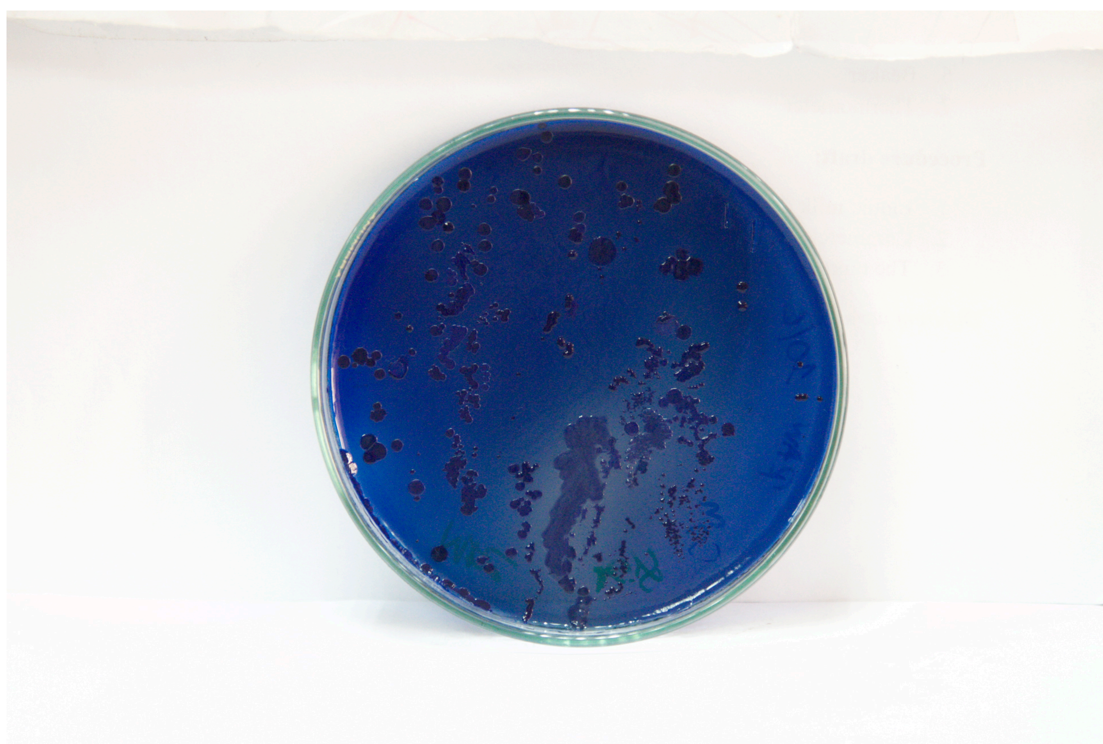

Figure 3.2 (c): Bacterial colonies on m-FCagar media. Blue colonies and colorless or light-blue colonies indicate lactose fermenter and non-fermenter respectively.

Table 3.9 (d): Colony characteristics of the isolates on MSA agar media

Collection Site	Isolate No	Colony characteristics				
		Size	Form	Color	Margin	Elevation
Site 1	22	Small	Circular	Yellow	Entire	Convex
	23	Medium	Circular	Pink	Undulate	Convex
Site 2	24	Medium	Circular	Pink	Entire	Raised
	25	Large	Circular	Yellow	Entire	Raised
Site 3	26	Large	Circular	Yellow	Entire	Convex
	27	Medium	Circular	Pink	Entire	Raised

Figure 3.2 (d): Bacterial colonies on MSAagar media. Yellow colonies and pink colonies indicate lactose fermenter and non-fermenter respectively.

Table 3.9 (e): Colony characteristics of the isolates on Cetrimide agar media.

Collection Site	Isolate No	Colony characteristics				
		Size	Form	Color	Margin	Elevation
Site 1	28	Small	Circular	Green	Entire	Raised
Site 2	29	Small	Circular	Colorless	Entire	Raised
Site 3	30	Medium	Circular	Green	Entire	Convex

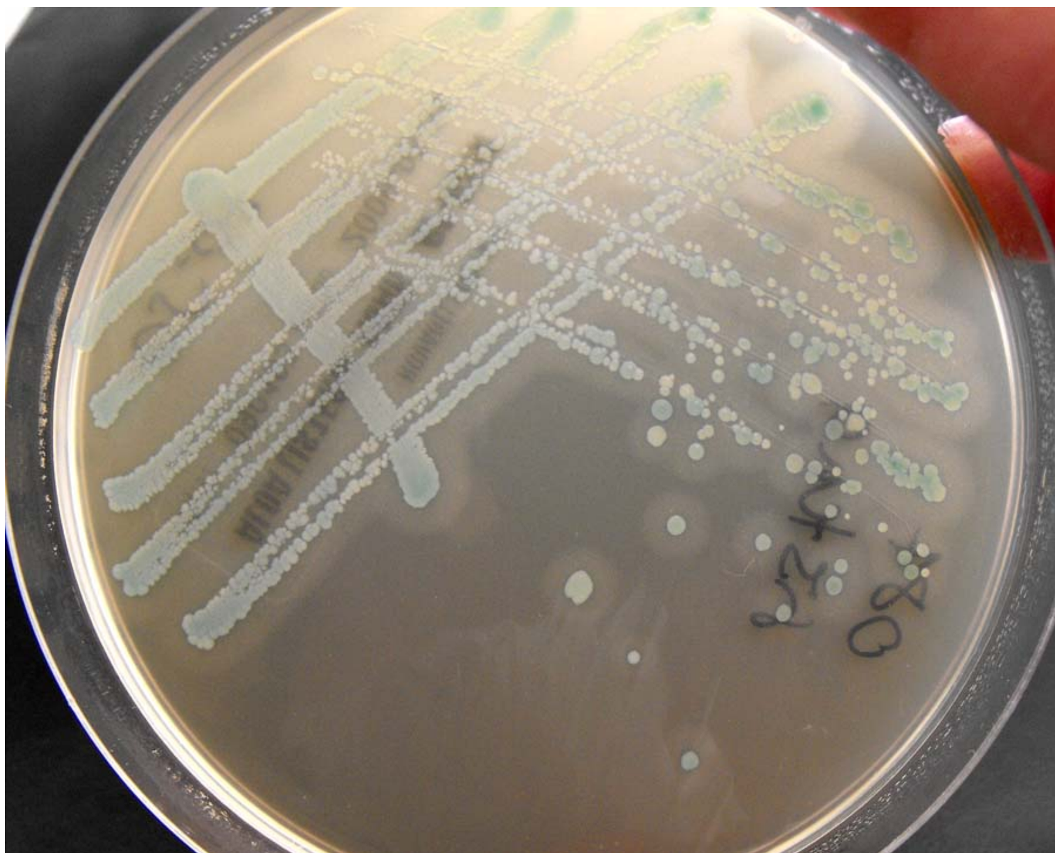

Figure 3.2 (e): Bacterial colonies on cetrimide agar media.

Table 3.10: Results of biochemical tests of the isolates collected from different agar media

Isolates	Biochemical tests													Presumptive organism
					TSI fermentation				Fermentation test					
	Indole production test	**Methyl red reactiontest**	**VogesProskeur'sreaction test**	**Citrate utilization test**	**Slant**	**Butt**	**CO_2**	**H_2S**	**Lactose**	**Sucrose**	**Dextrose**	**Catalase activity test**	**Oxidase activity test**	
1	-	+	-	-	A	A	-	-	-	A	A	+	-	*E. coli*
4	+	+	-	-	A	A	-	-	-	A	AG	+	-	*E. coli*
7	+	-	+	+	A	A	+	-	-	A	A	+	-	*Enterobacter sp.*
10	+	+	-	-	K	A	-	-	-	-	-	+	+	*V.parahemolytic*
12	+	+	-	+	A	A	-	-	-	-	-	+	+	*V.cholera*
15	+	+	-	+	A	A	-	+	-	-	-	+	+	*V.cholera*
13	+	+	-	-	A	A	-	-	-	-	AG	+	-	*V.mimicus*
24	+	+	+	-	A	A	-	-	-	A	A	+	-	*Staphylococcus aureus subsp.*
27	+	+	-	-	A	A	-	+	A	A	AG	+	-	*Staphylococcus sp.*
28	-	-	-	+	K	K	-	-	-	-	-	+	+	*P. aeruginosa*

K=Alkaline reaction, A=Acidic reaction, + =Positive reaction; - = Negative reaction

Figure 3.3 (a): Citrate utilization test
Left= Positive result. Right=Negative result

Figure 3.3(b): Urease production test in MIU medium
Left= Negative result. Right=Positive result

Figure 3.3 (c): Methyl red test in MR-VP broth
Left= Negative result. Right=Postive result

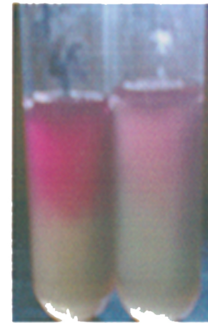

Figure 3.3 (d): Voges-Proskaure test in MR-VP broth
Left= Positive result. Right=Negative result

Figure 3.3 (e): Triple sugar iron test

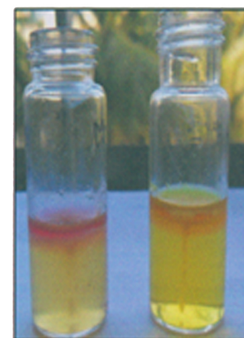

Figure 3.3(f): Indole production test MIU media
Left= Positive result. Right=Negative result

4. Discussion

Water is considered as an inevitable element of life. Around 75% of the earth is surrounded by water, but only 1% of water can be used as a source of drinking water for animal and human being. Our water sources include ground water, shallow ground water and surface water like pond, lake, river, rainwater etc but they are getting polluted with various organic and inorganic matters. The water sources are mostly contaminated with fecal wastes of poultry and livestock farms, sewage, pesticides, herbicides, industrial wastes, and biological agents such as bacteria, virus, fungus, protozoa etc. Consumption of contaminated water may cause various gastrointestinal diseases like diarrhea, dysentery and other water borne diseases like cholera, typhoid of human, poultry and livestock. The World Health Organization has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water or unavailability of water and at least 5 million deaths per year can be attributed to water born diseases (Karn et al., 2001).

The most common forms of freshwater habitats of Bangladesh are haor, baod, beel, lake, pond, rivers, and floodplains. The sediment/water phases are not permanently separated. Mineral and organic particles of the lake water, and enteric and other bacteria adsorbed by these particles, can pass to sediments column as a result of diving, rowing, walking in water, use of motor boats, navigation, sand extraction etc. Passage of bacteria from bottom sediment to water may also take place as a result of changes in salinity or concentration of organic matter. It is believed that enteric bacteria are able to metabolize elutes of bottom sediments, and develop and multiply in such aquatic ecosystems free of predators. In addition, survival of these microorganisms in surface is higher than in bottom sediments, and water may be a reservoir of these bacteria.

Gulshan-Baridhara Lake was declared an Ecologically Critical Area (ECA) in 2001. Continuation of all sorts of banned activities in the ECA has turned it into an ecologically dead lake. This has been manifested recurrently through the death of fishes and for vigorous media coverage each year. The water of the lake is being polluted day by day and during the summer season the level goes down and the pollution becomes worst. According to DWASA estimation, an average 12,000 cubic

meter of untreated waste mostly from the garments washing and dying plants from the Tejgaon, Badda and the Mohakhali industrial areas are disposed every day into the Gulshan Lake.

The present study was carried out to evaluate the microbiological condition of the water of Gulshan lake. The Gulshan lake is one of the fresh water lakes of Dhaka city. It is located in the Gulshan diplomatic area. People living around thelake use the lake water for various purposes like bathing, washing cloths, boat riding, fish cultivation etc. In addition, domestic waste waters of the surrounding residents are being introduced into the lake water. All of these activities contaminate the lake water. Some lake side shops use this lake water for washing their cooking utensils and sometimes as drinking water that might play role in the transmission of various water-borne diseases. It has been reported that the bacteria present in the water body gradually settle down to the sediment and thus the sediment serves as reservoir of bacteria (Droppo et al., 2009). Therefore, water samples were collected from different sites of Gulshan lake and were tested in the Laboratory of Microbiology, BRAC University to detect the presence of indicator and pathogenic bacteria. Water samples were inoculated onto five different selective media. *Escherichia coli*, *Enterobacter*, *Staphylococcus aureus*, *Pseudomonas and Vibrio species* were isolated. Bacterial count on MacConkey agar ranged between 1.3×10^3 cfu/ ml and 1.76×10^4 cfu/ml, where maximum count was observed in the sample of site1 during summer. The highest count of bacteria (2.17×10^4 cfu/100 ml) on m-FC agar was found in site 3 during rainy season, while the lowest count (4) was found in site1. Bacterial count on cetrimide agar ranged between 0 and 10cfu/ml during the whole time period. In TCBS agar the overall count was high. In MSA agar the count increased during the rainy season in all three sites .The physical properties of the water samples are given in above tables. In Summer, the water temperature ranged between 25 and 27°C, while in Rainy season that was around 29°C. The results indicate a favorable temperature for bacterial growth throughout the time period. The maximum pH (8.1) was detected in the site 3,while the minimum pH (7.6) was seen in the sample of site1. According to the United State Public Health (USPH), drinking water standards are pH 6.0 to 8.5DO (dissolved oxygen) was in a range between 3.5 to 5.5 mg/l. The identified community is a reflection of the partial community. The water quality parameters like TDS, conductivity, DO, BOD, pH have shown significant change during this time period. To some point they are not present as expecting by the nature to maintain our

ecosystem. Problem becomes more acute when dissolution of input in the environment exceeds the decomposition, dispersal, or recycling capabilities. The presence of different bacterial isolates indicate that the Gulshan lake water was polluted with chemical and bacterial pollutants. (Karimet al., 2012). After 2 years here the result is reflecting the same thing. The presence and abundance of *Escherichia coli, Aeromonas sp., Enterobacter sp., Pseudonomas sp.,* etc. in the water samples clearly showed significant level of microbial pollution of the lake (Saha et al., 2011).Ingestion of these enteric bacteria during bathing and swimming in the lake might cause intestinal diseases and somehow or rather entrance of waterborne pathogens in food chain around this area. Physico-chemical and bacteriological status indicated that the water of Gulshan lake is in critical stages of eutrophication. *Pseudomonas aeruginosa* has been employed as sewage indicator, while *Aeromonas hydrophila* as an indicator of eutrophication (Bahlaoui et al., 1997). In 1997, Godfree and coworkers mentioned that fecal streptococci is an indicator of fecal contamination in water. Indicator microorganisms, such as total coliform, fecal coliform and fecal streptococci have been used as a model for the potential presence of pathogenic microorganisms (Patra et al., 2009).

In conclusion, it can be said that Gulshan lake water serves as reservoirs of pathogenic bacteria. This study emphasizes the need for elaborate microbiological examinations of water from Gulshan lake and other lakes around Dhaka city. Dhaka city has been growing without much of plan and the city lacks systematic waste management system as well. The study was done created awareness regarding the environmental issues in Gulshan Lake. Various attempts should be taken to restore the water qualities of the Gulshan Lakelike - illegal encroachment and waste dumping should be stopped through implementation of existing laws and regulations.

5. References

- API-IA (American Public Health Association). (1995). Standard methods for the examination of water and waste water. 19th edition. pp. 1193. American Public Health Association, Washington, D.C.

- APHA (American Public Health Association). (1992). Standard methods for the examination of water and waste water. l8 edition. American Public Health Association, Washington, D.C.

- APHA (1998). Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association, Washington, D.C.

- Atlas, R., M. (1997). Bacterial diversity. In: Principles of microbiology, Fishback, J., E. Editor, 2 ed. Boston, Mass.: Wm. C. Brown Publishers.

- Ainsworth, R. (2004) Safe piped water: Managing microbial water quality in piped distribution systems. Geneva, TWA Publishing, London, for the World Health Organization.

- Albert, M. J., Qadri, F., Haque, A. &Bhuiyan, N. A. (1993). Bacterial clump formation at the surface of liquid culture as a rapid test for identification of enteroaggregativeEseherichia coli.JClinMicrobiol 31: pp.1397-1399.

- Auerbach, P., S., Yajko, D., M., Nassos, P., S., Kizer, K., W., Morris, J., A., Jr. & Hadley, W., K. (1987). Bacteriology of the freshwater environment implications for clinical therapy. Ann EmergMedl6: 1016-1022.

- Bangladesh Health and Injury Survey Report on Children, Dhaka, Bangladesh. (2005).

- Beneson, A., S. (1995). Control of Communicable Diseases Manual,l6th ed. American Public Health Association, Washington, D.C.

- Burton, Jr., G., A., Gunnison, D. &Lanzai, G. (1987). Survival of pathogcnibacWria in various freshwater sediments.Appi Environ Microbiol 53. (4).pp 633-638.

- Karim.Md. Abdul., Nazneen. A and Sirajul. H.(2012). bacterial status and pollution level in the water of Gulshan lake, Dhaka. *Bangladesh J. Zool*. Vol.40.(2).pp 165-173.
- Bilotta, G., S., Brazier, R., E. &Haygarth, P., M. (2007). The Impacts of Grazing Animals on the Quality of Soils, Vegetation, and Surface Waters in Intensively Managed Grasslands. AdvAgron 94: 23 7-280.
- Cappuccino, J.G. & Sherman, N. (1999). In Microbiology: A Laboratory Manual, 4th ed, pp 199—204. Addison Wesley Longman, Inc. Harlow, England
- Clark, M., A., & Barrett, E., L. (1987). The phs gene and hydrogen sulfide production by Salmonella typhimurium. .1 Bacteriol 169:2391-2397
- Doetsch, R. N. (1981). Determinative methods of light microscopy. In Manual of Methods for General Microbiology, pp. 21-33. Edited by Gerhardt, P. et a!. American Society for Microbiology.
- Droppo, I., G., Liss, S. N., Williams, D., Nelson T., Jaskot. C & Trapp B. (2009). Dynamic existence of waterbome pathogens within river sediment compartments.
- Implications for water quality regulatory affairs. Environ SciTechnol 43(6): 1737-43.
- Donlan, R., M. (2001). Biofllm and device-associated infections (Special Issue). Emerg infect Dis 7(2): 277-28 1.
- Graevenitz, A.V. (1985). Aeromonas and Plesiomonas. In Lenette, E.H., Balows, A., Hausler, W.J., Shadomy, H.J. Manual of Clinical Microbiology, 4t ed. pp. 278-281. American Society for Microbiology.Washington, D.C.
- Gerba. C.. P. &Mcleod, J., S. (1976). Effect of sediments on the survival of Lscherichia coil in marine waters. Appi Environ Microbiol 32: 114.
- Hossain. M.. Z., Kabir, G., M., A. &Quraishi, S., B. (2010). Contamination of Dlianmondi and Guishanlake waters with some metals and inorganic pollutants. Jóurmzl of Bangladesh Academy of Sciences 34(1): 89-93.

❖ Karn. S... K. & Harada, H. (2001). Surface water pollution in three urban territories of Nepal. India. and Bangladesh. Environmental Manage 28 (4): 483-96.

❖ Khan. M.. R.. Saha, M., L. & Ciboria, A., H., M., G. (1992). A bacteriological profile of bottled water sold in. World Microbial Biotechnology 8(5). 544-545.

❖ MJ Pelzer & RD Reid (1978). Microbiology, 3d end. New York: McGraw-Hill Phasing.

❖ Niewolak, S. (1998). Total Viable Count and Concentration of Enteric Bacteria in Bottom Sediments from the CzamaHañczaRiver , Northeast Poland. Polish Journal of Environmental Studies **7(5):** 295-306.

❖ O'Connor, T., G., Heron, J., Golding, J., Beveridge, M. & Glover, V. (2002). Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. Br JPsychiatry 180: 502-508.

❖ Pinaki, R. (2004). Environmental articles archive: Dying rivers deliver deluge blow.Available at: http://www.bcas.net/EnvFeatures/Land/2OO5/April2005.html. AccessedJune 20, 2011.

❖ Stoodley, L., H. &Stoodley, P. (2005). Biofllm formation and dispersal and the transmission of human pathogens. Trends Microbiol, **13(1):** 7-10.Swapan, K., N. (2005). Shigellosis. JMicrobiol**23(2):** 133-143.

❖ vanHoudt, R., Aertsen, A., Jansen, A., Quintana, A., L. &Michiels, C., W. (2004). Biofilm formation and cell-to-cell signalling in Gram-negative bacteria isolated from a food processing environment. I App!.Microbiol**96 (1):** 177-184.

❖ Waterbome Pathogens. Available at: http://www.simondawson.com/bugch2.html. Accessed June 23, 2011.

❖ Shah.M. L. M.Nessa. M. R. KHAN.(2012). Bacteriological and physicochemical water quality of four ponds of Dhaka metropolis. *Bangladesh J. Bot*. 41.(1).pp. 55-60, 2012.

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°C for 15 min.

1. **Nutrient Agar (Himedia, India)**

Ingredients	Amounts (g/L)
Peptic digest of animal tissue	5.0
Beef extract	1.50
Sodium chloride	5.0
Yeast extract	1.50
Agar	15.0

2.M-FC Agar Base (Himedia, India)

Ingredients	Amounts (g/L)
Tryptose	10.0
Proteose peptone	5.0
Yeast extract	3.0
Lactose	12.50
Bile salts mixture	1.50
Sodium chloride	5.0
Aniline blue	0.10
Agar	15.0

3. Nutrient Broth (Oxoid, England)

Ingredients	Amount (g/L)
Lab-lemco powder	**1.0**
Yeast extract	**2.0**
Peptone	**5.0**
Sodium chloride	**5.0**

4. Cetrimide agar (Merck, India)

Ingredients	Amount (g/L)
Pancreatic digest of gelatin	**20.0**
Magnesium chloride hexahydrate	**1.4**
Potassium sulfate anhydrous	**10.0**
Cetrimide	**0.3**
Agar-Agar	**13.0**

5. T_1N_1 soft agar

Ingredients	Amount (g/L)
Tryptone	**0.6 g**
Sodium chloride	**0.3g**
Agar	**0.42 g**

6. Tryptone soy broth, (Oxoid, England)

Ingredients	Amount (g/L)
Pancreatic digest of Casein	**17.0**
Papaic digest of soybean meal	**3.0**
Sodium chloride	**5.0**
Di-basic potassium phosphate	**2.5**
Glucose	**2.5**

7. **MacConkey agar (Oxoid, England)**

Ingredients	Amount (g/L)
Peptone	**20.0**
Lactose	**10.0**
Bile salts	**5.0**
Sodium chloride	**5.0**
Neutral red	**0.075**
Agar	**12.0**

8. **Simmon's citrate agar (Oxoid, England)**

Ingredients	Amount (g/L)
Magnesium sulfate	**0.2**
Ammonium dihydrogen phosphate	**0.2**
Ammonium phosphate	**0.8**
Sodium citrate	**2.0**
Sodium chloride	**5.0**
Agar	**15.0**
Bacto brom thymol blue	**0.08**

9. **Peptone Water**

Ingredients	Amount (g/L)
Peptone	**10.0**
Sodium chloride	**5.0**

10. **MR-VP broth**

Ingredients	Amount (g/L)
Peptone	**7 g**
Dextrose	**5 g**
Potassium phosphate	**5 g**

11. Triple sugar iron agar (Himedia, India)

Ingredients	Amount (g/L)
Peptic digest of animal tissue	**10.0**
Sodium chloride	**5.0**
Lactose	**10.0**
Sucrose	**10.0**
Dextrose	**1.0**
Ferrous sulfate	**0.20**
Sodium thiosulfate	**0.30**
Casein enzymatic hydrolysate	**10.0**
Yeast extract	**3.0**
Beef extract	**3.0**

12. Mannitol Salt agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	**10.0**
Manitol	**10.0**
Lab-lemco powder	**1.0**
Sodium chloride	**75.0**
Phenol red	**0.025**
Agar	**15.0**

13. Thiosulfate Citrate Bile Salts Sucrose agar (Difco, USA)

Ingredients	Amount (g/L)
Proteose peptone	**10.0**
Sodium thiosulfate	**10.0**
Sodium citrate	**10.0**
Yeast extract	**5.0**
Oxgall	**8.0**
Sucrose	**20.0**
Sodium chloride	**10.0**

Ferric citrate	**1.0**
Bromothymol blue	**0.04**
Thymol blue	**0.04**
Agar	**15.0**

14. Phenol red (Lactose, Dextrose, Sucrose) Broth

Ingredients	Amount (g/L)
Trypticase	**0.4**
Lactose	**0.2**
Sucrose	**0.2**
Dextrose	**0.2**
Sodium chloride	**0.2**
Phenol red	**0.00072**
Final pH	7.3

APPENDIX-II

Buffers and reagents

1. Phosphate buffered saline (PBS)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 2.0 g of KH_2PO_4 in 800 ml of distilled water. The pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1 liter by distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

2. Kovac's reagent

5 g of para-dimethylaminobenzaldehyde was dissolved in 75 ml of amyl alcohol. Then concentrated HCl was added to make the final volume 25 ml. This reagent was covered with aluminum foil and stored at 4^oC.

3. Methyl red reagent

0.1 g of methyl red was dissolved in 300 ml of 95% ethyl alcohol. Then distilled water was added to make the final volume 500 ml. This reagent was covered with aluminum foil and stored at 4^oC.

4. Barritt's reagent

Solution A

5 g of alpha-naphthol was dissolved in 95% ethanol. This solution was covered with aluminum foil and stored at 4^oC.

Solution B

40 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. This solution was covered with aluminum foil and stored at

5. 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°C.

APPENDIX-III

Instruments

The important equipments used through the study are listed below:

Autoclave	SAARC
Freeze (-20°C)	Siemens
Incubator	SAARC
Micropipette (10-100µl)	Eppendorf, Germany
Micropipette (20-200µl)	Eppendorf, Germany
Oven, Model:MH6548SR	LG, China
pH meter, Model: E-201-C	Shanghai Ruosuaa Technology company, China
Refrigerator (4°C), Model: 0636	Samsung
Safety cabinet Class II Microbiological	SAARC
Shaking Incubator, Model: WIS-20R	Daihan Scientific, Korea
Vortex Mixture	VWR International
Water bath	Korea
Weighing balance	ADAM EQUIPMENT™, United Kingdom
Lutron conductivity meter cd-4302	Lutron Electronic Enterprise Co., Ltd. Taiwan
Lutron dissolved oxygen meter do-5509	Lutron Electronic Enterprise Co., Ltd. Taiwan
TDS meter DiST 2	Hanna instroments