**Molecular detection of the seasonal bacterial diversity from a freshwater lake in West Bengal, INDIA**

**Abstract:**

Situated in the northeastern region of Kolkata, WB, Subhas Sarobar is a medium-sized artificial lake that is home to a variety of plants and animals, such as benthos, nekton, zooplankton, bacteria, and phytoplankton, all of which contribute to a balanced and interdependent ecosystem. The water's physic-chemical characteristics are impacted by seasonal variations in temperature, precipitation, and humidity, which could be a factor in the change in the population of fauna. In addition, this aquatic system uses wastewater from homes. Therefore, in addition to seasonal fluctuations, the physico-chemical properties of the lake water are impacted by domestic discharges, washing clothing and utensils, bathing, and disposal of plastic garbage near the water. All of these human activities eventually lead to a decline in the lake's water quality. The lake system's low DO and high nutrient content create an ideal habitat for the growth of bacteria, including those that are infectious and could harm public health and other aquatic species. Containing harmful bacteria species in water is dangerous and can lead to a number of health problems, including diarrhea and gastroenteritis with fever, chills, nausea, hypertensive septic shock, secondary lesions, and infection. It may be possible to successfully stop the spread of disease if the traits and specifics of pathogenic bacteria are understood. Several enteropathogenic bacteria, including Shigella, Vibrio, Cholera, Salmonella, Enterobacter, and Yersinia, were identified, isolated, and purified in this study. In a model of live and heat-killed mice, the spread of bacterial zoonotic infections to other animals was found to cause major public health issues for those who are directly or indirectly using this water.

**Kew words:**

Biological tools & techniques, Water quality, Bacterial diversity, Enteropathogenic bacteria, Public Health, Subhas Sarobar, West Bengal.

**1. Introduction:**

The oxygen balance is maintained in lakes, making them the most productive and life-sustaining aquatic habitat. The water cycle and the ecosystem are typically regulated by lakes. In general, freshwater in urban areas has recreational and aesthetic value. Aquatic vegetation, mostly macrophytes from submerged, floating, and emergent categories, makes up the lake ecosystem (Saha, 2000). It offers an appropriate surface area for protection, oviposition sites, development, and nesting grounds for macroinvertebrates, Pisces, shellfish, and other aquatic aminals. As "microcosms," or tiny intra-universes, lake ecosystems respond to a wide range of variations in their floral and faunal compositions, which are highly dynamic (Saha and Basak, 2024). As a result, limnological methods for studying lakes aid in the evolution of ecosystem ecology as a habitat. Through macrophytic plants, the lake ecology plays a crucial part in naturally preserving the oxygen balance. In 1965, on the 69th anniversary of Netaji Subhas Chandra Bose's birth, Subhas Sarovar, the first artificial lake to be excavated as a recreational area during the 2nd Five Year Plan, was created for recreational purposes. It is located at latitude 220 34′ - 220 34′ 30′′ N and longitude 880 24′ - 880 24′ 30′′ E. Of the Subhas Sarobar's 39.6 hectares total area, about 6.04 ha are submerged in water (Basak *et al.*, 2024). Two islands, one small and the other huge, provide an excellent habitat for a wide range of organisms. The lake's mean length and width are 0.584 kilometers and 0.326 kilometers, respectively, while its maximum and mean depths are 10.36 meters and 9.6 meters. This lake, which is around 98 acres in total size (including the water bodies), was designed by the C.I.T. to provide infill material for the construction of this road network (Khan and Sen, 2002). About 40 acres of the 100 acres of land were excavated to make the lake. East Calcutta's lungs are Subhas Sarovar or Subhas Lake, whose ecosystem serves as a natural sink by drawing toxins out of the surrounding area. Thus, this lake is crucial to preserving the environment of the city. The two islands and the large body of water, along with the natural surroundings, enhance the biological diversity of the area (Samal *et al.*, 2004). The emergence of the bacterial population and the physico-chemical characteristics of the lake's habitat is being impacted by everyday activities such as bathing, laundry, nutrient input, high plankton, etc. Many causes of water pollution, such as wastes and byproducts, cause natural ecosystems to become contaminated with bacteria and other pathogens, and human use of the water is also impacted. Human activities have been putting a lot of strain on the Subhas Sarobar recently. The facility is used by approximately 3,000 people every day for bathing and washing clothes and utensils (Basak *et al.*, 2024). Besides the lake, there is also a solid waste disposal facility that handles plastic. The conditions of the environment, particularly the lake water quality, have gotten worse as a result of all these human activities (Saha *et al.*, 2001). The sustainable development of the area's land and aquatic ecosystems is thus threatened by environmental degradation.

* 1. **Physical Status of Freshwater Lake:**

The Lake environment is a dynamic aquatic ecosystem with a wide variety of plants and animals (Figure 1). The Calcutta Improvement Trust oversees Subhas Sarobar, a man-made lake that serves as East Calcutta's lung and gains a lot from the natural surroundings (Figure 1). Its longitude ranges from 880 24′ to 880 24′ 30′′, while its latitude ranges from 220 34′ to 220 34′ 30′′ N. Water covers about 6.04 ha of the Subhas Sarobar's 39.6 ha total area. A great home for many different types of life is made up of two islands, one little and the other enormous. According to CMC 1986, the lake and surrounding vegetation make up 39.6 hectares of the total area, of which 16.29 ha constitute the water body. Due to seasonal variations throughout the year, the water body's total volume averages 634 m3, and its mean depth is 4.8 m (Samal *et al.*, 2004).

**1.2. Physicochemical status of Freshwater Lake:**

The following parameters were measured and recorded: temperature (Hanna, Romania); pH (Hanna, Romania); dissolved oxygen (Lutron, Taiwan); salinity (Erma, Japan); ammonia (Himedia kit); nitrite (Himedia kit); nitrate (Himedia kit); hardness (Himedia kit); and Vibrio sp. (Himedia kit). This allowed for the immediate assessment of the health status of the water in the cultured pond and the collection of water in bottles to identify the bacterial population and species. Five sampling sites were chosen for the monthly collection of water samples to assess the water quality during the study period. Surface water samples were obtained by carefully wading one-liter polythene cans into the water. The conventional procedures (APHA, 1992; Saha, 2000) were used to finish the analysis (Table 1).

**Table 1: Physicochemical Factors** (Adapted from **Khan and Sen, 2002**)

|  |  |
| --- | --- |
| **Physicochemical Factors** | |
| Water temperature(0C) | 30.20C |
| pH | 8.24 |
| Turbidity | 16.42 |
| Conductivity | 417.37 |
| Dissolved Oxygen (ppm) | 5.7 |
| Total alkalinity(mg/lit) | 216.47 |
| Chloride (mg/l) | 42.82 |
| Phosphate (mg/l) | 0.04 |
| Nitrite (mg/l) | 0.12 |
| Nitrate (mg/l) | 0.29 |
| Ammonium (mg/l) | 0.20 |



**Figure: 1. Areas of Subhas Sarobar Lake for collection of water samples (5 sites).**

* 1. **Zooplankton Status**: The water body was home to a diverse array of zooplankton, with the most common being cladocerans, which are followed by copepods and rotifers. The following is a list of several zooplanktons (Saha et al., 2001) (Table 1).
  2. **Table 2: List of few Zooplanktons** (Adapted from Khan *et al.*, 2002)

|  |  |
| --- | --- |
| **Group** | **Name of Zooplankton** |
| Copepoda | *Cyclops* sp.  *Mesocyclops* sp.  *Heliodiaptomus* sp. |
| Rotifer | *Brachionus* sp.  *Testudinella* sp.  *Lacane* sp. |
| Cladocera | *Sida* sp.  *Moina* sp.  *Allona* sp.  *Pseudosida* sp. |

**1.4. Bacterial Diversity of The Lake**:

In recent years, the negative consequences of human activity have significantly increased. Subhas Sarabor is a eutrophic lake due to the high concentration of nutrients it receives from the environment as a result of human activities. In addition to increasing the populations of algae and phytoplankton in the lake, these nutrients also reduce the amount of oxygen available to other aquatic life. Numerous harmful bacteria that can cause severe waterborne illnesses in people can arise from this condition of the water body (Dutta et al., 2018; Saha et al., 2024) (Table 3). Salmonella, which causes typhoid fever, Shigella, which causes diarrhea, Vibrio, which causes regular diarrhea and sudden nausea and vomiting, Escherichia, which causes urinary tract infections, and Yersinia, which causes gastrointestinal diseases ranging from mild diarrhea to inflammation of the mesenteric glands), Tetanus (food poisoning), Aeromonas (Gastroenteritis) (Cabral, 2010).

**Table 3: List of few pathogenic bacteria**

|  |  |
| --- | --- |
| **Diseases** | **Causative Bacteria** |
| Cholera | *Vibrio cholerae* |
| Gastroenteritis caused by vibrios | *Vibrio parahaemolyticus* |
| Typhoid fever and other serious salmonellosis | *Salmonella* sp. |
| Bacillary dysentery or shigellosis | *Shigella* sp. |
| Acute diarrheas and gastroenteritis | *Escherichia coli* |
| Gastroenteritis | *Aeromonas* sp. |

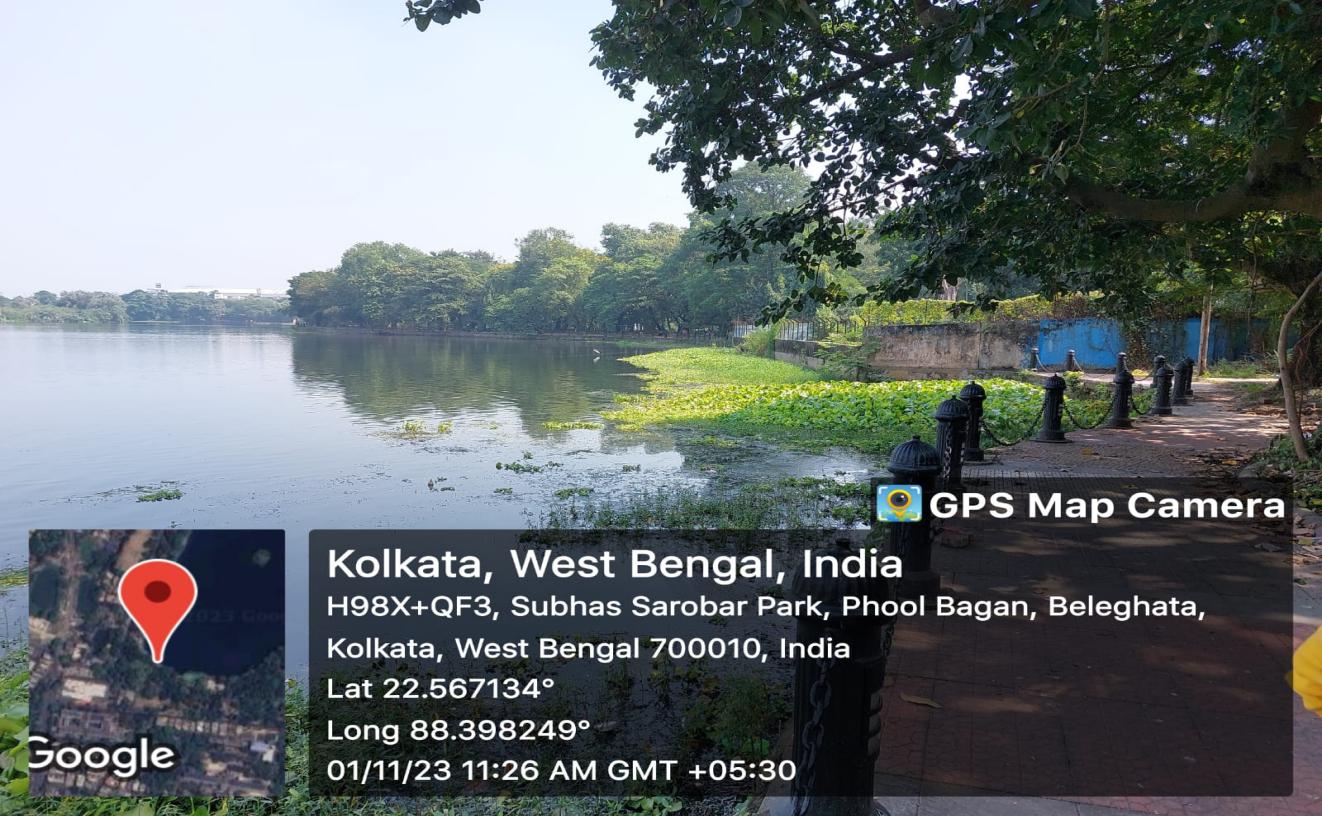
**1.5. Factors Responsible for The Lake Pollution**:

One of the biggest problems on the lake's grounds has been the unrestricted access to the lake and its natural resources by the nearby slum dwellers (Khan and Sen, 2002). The poor settlements' dependence on the lake's water and nearby resources for everyday necessities has grown as a result of the lack of infrastructure. This includes bathing, fishing in the lake, peeing around its edge, cleaning different cars with the water, dumping rubbish in this body of water, and regularly using the lake's meager water supply to wash clothes and cutlery. One of the primary contaminants of lake water is nonylphenol, which is found in detergents used to clean clothing and cutlery. The lake water is also being contaminated by other substances, such as microplastics and excrement (Khan and Sen, 2002). These pollutants pose major risks to human health because they bioaccumulate and get into the human food chain through fish. Phosphate salts, which are abundant in laundry detergents, can contaminate water. This phosphate salt makes the water body eutrophic by stopping organic materials from biodegrading. Eutrophication causes an algal bloom and raises the BOD level, which kills aquatic life. Since people use the lake water on a daily basis, all of these factors combined also encourage the growth of dangerous waterborne bacteria, which poses a major risk to human health (Khan and Sen, 2002).

**2. Material and Methods**:

**2.1. Site Selection –**

Water and tissue samples (blood) were collected from five different sites of Subhas Sarobar throughout the year (November 23 to October 2024). The schedule was prepared after collecting information from journals, and articles and after completing pilot field surveys in different sites of Subhas Sarobar Kolkata during pre-monsoon, monsoon, and post-monsoon physical analyses (**Figure 2**). Several aquatic animals (fish and shellfish) and water were collected during the survey for the detection of temperature, pH, dissolved oxygen, salinity, TDS, and nitrogen content (**Saha *et al.*, 2022**). Blood and other organs were examined in aquatic animals (fish and shellfish) to detect bacterial load, infection, and diversity.



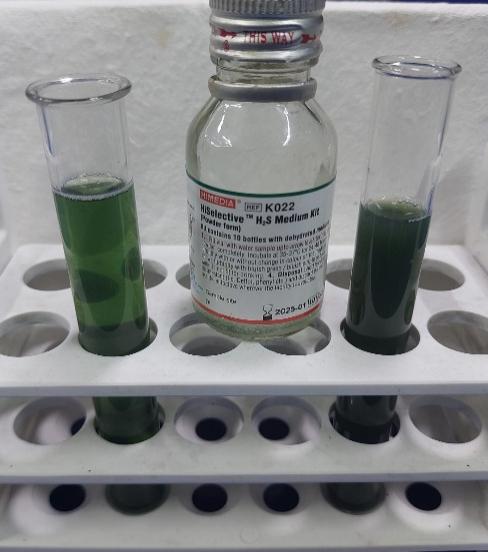
**Figure:2.** Water sample collection in Subhas Sarobar

**2.2. Determination of Water Quality –**

The following parameters were measured and recorded: temperature (Hanna, Romania); pH (Hanna, Romania); dissolved oxygen (Lutron, Taiwan); salinity (Erma, Japan); ammonia (Himedia kit); nitrite (Himedia kit); nitrate (Himedia kit); hardness (Himedia kit); and Vibrio sp. (Himedia kit). This allowed for the immediate assessment of the health status of the water in the cultured pond and the collection of water in bottles to identify the bacterial population and species. Five sampling sites were chosen for the monthly collection of water samples to assess the water quality during the study period. Surface water samples were obtained by carefully wading one-liter polythene cans into the water. The standard procedures were used to finish the analysis (APHA, 1992).

**2.3. Physical Detection of Bacteria (Kit Method):**

A bacterial identification bottle kit was filled with collected water from the lake (Figure 3). The bottle should be incubated at 350C for 24 to 48 hours. The color of bacterial colonies makes it easier to identify the various bacteria present in lake water, which range from green to red, pink, blue, and black (Figure 2).



**Fig: 3. Detection of the bacterial population by colour using the kit (Himedia)**

Lake water samples were gathered, and using the spread plate method, at least 0.1 ml of the water sample was placed on species-specific microbiological culture media. After a 24-hour incubation period at room temperature (350C) in a bacteriological incubator, examine the bacterial colonies. Bacterial contamination of water with bacterial species is shown by the presence of color or bacterial colonies (droplets) on plates. The average number of colonies per plate was multiplied by the reciprocal of the dilution factor to determine the counts of colonies per milliliter. Colony-forming units (CFU) per milliliter of sample are the unit of measurement used to express the computed results. Colony Forming Units (CFU) per 100 µl of blood (CFU/100 µl) were counted three times for each colony, sorted by size and color, and then averaged. For purification and characterisation, distinct individuals of every colony type were then chosen at random (Saha and Dash, 2021; Basak et al., 2024).

**2.4. Molecular Detection:**

**2.4.1. DNA extraction:**

Selected colonies from the medium agar were moved onto tryptone soy agar and, following a 24-hour incubation period at 350C, purified into TSB. The cultured bacterial samples must be centrifuged and moved into a pellet in the bottom of a micro centrifuge tube following final purification and growth in TSB. Following the manufacturer's instructions, a bacterial genomic DNA isolation kit was used to isolate the genomic DNA from the bacterial pellet. Following centrifugation and a 70% ethanol wash, the DNA pellet was dissolved in TE buffer and then stored at -200C for further use. The quality and quantity of isolated genomic DNA for each sample are to be evaluated by measuring and calculating the ratio of optical densities at 260 nm and 280 nm wavelengths respectively, using a NanoDropTM spectrophotometer. The aqueous phase contains purified DNA and is to be directly used for subsequent experiments (Saha and Dash, 2021; Saha Basak, 2024).

**2.4.2. PCR Amplification and Agarose gel electrophoresis and documentation:**

One hundred nanograms of genomic DNA should be used as a template for the PCR reaction. Phusion reaction buffer with a final concentration of 1 X, 0.2 U of PhusionTM high fidelity DNA polymerase, 200 µM dNTPs, forward and reverse primers with final concentrations of 0.5 µM each, and template DNA were all included in each reaction mix. The literature that is currently available is used to choose primers (Mookherjee et al., 2015) (Table 5). A heat cycler (Gene Amp 9700, ABI) was used to carry out the amplification. The amplified products must be examined on 1%agarose gels containing 0.5 µg/ml ethidium bromide in 1X Tris-acetate-EDTA (TAE) solution following the completion of the PCR amplification stages. Following electrophoresis, the gel must be inspected and pictures must be taken for the gel documentation system (Saha *et al*., 2023).

**Table: 4. Primer selection of bacteria for freshwater bodies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name of Primer** | **Sequence** | **Product**  **Length (bp)** | **Annealing**  **Temp.** | **Targets sp** |
| 8F  1492R | 5’-AGAGTTTGATCCTGGCTCAG-3'  5’-GGTTACCTTGTTACGACTT-3' | **1600** | 500C  1 min | Universal primer for bacteria |
| 27F  1492R | 5’- AGAGTTTGATCMTGGCTCAG-3’  5’- TACGGYTACCTTGTTACGACTT-3’ | **1500** | 500C  1 min |
| 700F  1325R | 5’-CGGTGAAATGCGTAGAGAT-3’  5’-TTACTAGCGATTCCGAGTTC-3’ | **663** | 570C  45 S | *Vibrio* genus  (16srRNA) |
| Vc-F  Vc-R | GTTCGCGCTGGTGAAGGTTCA TGGCATACCAGAGTCTTTCTGTG | **192** | 570C  1 min | ***Vibrio cholerae*** |
| 16S rDNA-F  16S rDNA-R | 5’-AGAGTTTGATCATGGCTTACGACTT-3’  5’-GGTTACCTTGTTACGACTT-3’ | **1542** | 550C  30 sec | ***Enterobacter* sp.** |
| uidA-F  uidA-R | 5’-AAAACGGCAAGAAAAAGCAG-3’  5’-ACGCGTGGTTAACAGTCTTGCG-3’ | **371** | 550C  30 sec | ***E.coli*** |
| Aero-F  Aero-R | 5’-TGTCGGSGATGACATGGAYGTG-3’ 5’-CCAGTTCCAGTCCCACCACTTCA-3’ | **720** | 550C  30 sec | ***Aeromonas hydrophila*** |
| IpaB-F  IpaB-R | 5′-GGACTTTTTAAAAGCGGC GG-3′  5′-GCCTCTCCCAGAGCCGTC TGG-3′ | **314** | 550C  30 sec | ***Salmonella*** ***enterica*** |
| IpaH-F  IpaH-R | 5’-TGGAAAAACTCAGTGCCTCT-3’ 5’-CCAGTCCGTAAATTCATTCT-3’ | **423** | 550C  30 sec | ***Shigella* sp.** |
| Ail-F  Ail-R | 5’-CTATTGGTTATGCGCAAAGC-3’ 5’-TGGAAGTGGGTTGAATTGC-3’ | **354** | 570C  30 sec | ***Yersinia enterocolitica*** |
| HP-F  HP-R | 5′-TCTGTCTGATTCGCTTTTCTG-3′  5′-AAGCTCGCTAAAAACGACC-3′ | **132** | 540C  5 Sec | ***Helicobacter pylori*** |

**3. Result:**

**3.1. Water Quality Parameter Statistics:**

Water quality parameters were recorded in the following tables to correlate the impact of different stressors leading to disease occurrences. The various water quality parameters like temperature, salinity, hydrogen ion concentration (pH), alkalinity, dissolved oxygen (DO), TDS, ammonia, nitrite, nitrate, etc. of the water determine the survival and multiplication of the bacterial population (Basak *et al.*, 2024). The main reasons for bacterial outbreaks are high organic loads, bad water conditions, and immunological suppression of hosts. Such water parameters influence the abundance of the bacterial population.

**Table: 5. Water parameters of Subhas Sarobar from January to December 2022 (Kolkata, WB)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Water Parameters** |  | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** |
| **Temperature (0C)** | 34±4 | 34.5±4 | 34.2±5 | 32.5±5 | 32.2±5 |
| **pH** | 8.7±3 | 8.2±2 | 8.5±3 | 8.5±4 | 8.2±3 |
| **Salinity (ppt)** | 0.0106±0.21 | 0.0106±0.24 | 0.0105±0.20 | 0.0106±0.24 | 0.0105±0.21 |
| **Alkalinity (ppm)** | 125±25 | 139±30 | 147±26 | 135±27 | 141±29 |
| **DO2 (mg / L)** | 5.3±1.4 | 6.1±3 | 6.5±1.8 | 5.7±2.8 | 5.8±4 |
| **DCO2 (mg / L)** | 8±2 | 9±2.5 | 8±3 | 9±4.1 | 9±3.9 |
| **TDS (ppm)** | 160±30 | 148±26 | 150±28 | 155±18 | 160±28 |
| **Ammonia (mg / L)** | 0.5±0 | 0.5±0 | 0.5±0 | 0.5±0 | 0.5±0 |
| **Nitrite (mg/L)** | 0.5±0 | 0.5±0 | 0.5±0 | 0.5±0 | 0.5±0 |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 |
| **Bacteria (+ / -) by kit** | +++ | +++ | +++ | +++ | +++ |

**Table:6. Water parameters of Subhas Sarobar on August & September, 23 (Kolkata, WB)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: August & September, 23** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature (0C)** | 34 | 34.5 | 34.2 | 32.5 | 32.2 | **33.34** |
| **pH** | 8.7 | 8.2 | 8.5 | 8.5 | 8.2 | **8.42** |
| **Salinity (ppt)** | 0.0106 | 0.0106 | 0.0105 | 0.0106 | 0.0105 | **0.0105** |
| **Alkalinity (ppm)** | 125 | 139 | 147 | 135 | 141 | **137.4** |
| **DO2 (mg / L)** | 5.3 | 6.1 | 6.5 | 5.7 | 5.8 | **5.88** |
| **DCO2 (mg / L)** | 8 | 9 | 8 | 9 | 9 | **8.6** |
| **TDS (ppm)** | 160 | 148 | 150 | 155 | 160 | **154.6** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -) by kit** | + | + | + | + | + | + |

**Table: 7. Water parameters of Subhas Sarobar on October & November, 23 (Kolkata, WB)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: October & November, 23** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature** | 29.6 | 31.6 | 30.5 | 32 | 30.2 | **30.78** |
| **pH** | 6.5 | 8.8 | 8.5 | 7.9 | 8.2 | **7.98** |
| **Salinity (ppt)** | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | **0.010** |
| **Alkalinity (ppm)** | 125 | 135 | 140 | 130 | 140 | **134** |
| **DO2 (mg / L)** | 5.8 | 4.6 | 6.8 | 5.7 | 6.2 | **5.82** |
| **DCO2 (mg / L)** | 9 | 9 | 8 | 9 | 8 | **8.6** |
| **TDS (ppm)** | 171 | 217 | 171 | 180 | 180 | **183.8** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -) by kit** | + | + | + | + | + | + |

**Table: 8. Water parameters of Subhas Sarobar on December & January, 23 (Kolkata, WB)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: December & January, 2023** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature** | 25.8 | 24.8 | 24.3 | 25.1 | 24.8 | **24.96** |
| **pH** | 7.3 | 7.4 | 8.1 | 7.8 | 7.4 | **7.6** |
| **Salinity (ppt)** | 0.011 | 0.010 | 0.010 | 0.011 | 0.011 | **0.010** |
| **Alkalinity (ppm)** | 118 | 125 | 139 | 130 | 140 | **130.4** |
| **DO2 (mg / L)** | 5.8 | 5.7 | 6.2 | 5.8 | 5.7 | **5.84** |
| **DCO2 (mg / L)** | 8 | 9 | 9 | 9 | 9 | **8.8** |
| **TDS (ppm)** | 220 | 171 | 217 | 171 | 180 | **191.8** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -) by kit** | + | + | + | + | + | + |

**Table: 9. Water parameters of Subhas Sarobar on February & March, 24 (Kolkata, WB)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: February & March, 2024** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature** | 20.2 | 21 | 20.9 | 21.9 | 21.8 | **21.2** |
| **pH** | 10.2 | 7.4 | 7.4 | 7.6 | 8 | **8.1** |
| **Salinity (ppt)** | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | **0.02** |
| **Alkalinity (ppm)** | 125 | 123 | 120 | 130 | 140 | **127.6** |
| **DO2 (mg / L)** | 3.8 | 3.7 | 10.5 | 8.5 | 9 | **7.1** |
| **DCO2 (mg / L)** | 09 | 09 | 08 | 09 | 08 | **8.6** |
| **TDS (ppm)** | 228 | 188 | 188 | 197 | 173 | **194.8** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -)** | + | + | + | + | + | **+** |

**Table: 10. Water parameters of Subhas Sarobar on April & May, 24 (Kolkata, WB)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: April & May, 2024** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature** | 29.0 | 29.6 | 28.6 | 28.5 | 29.1 | **28.9** |
| **pH** | 7.5 | 8.4 | 7.5 | 8.4 | 7.5 | **7.81** |
| **Salinity (ppt)** | 0.0106 | 0.0106 | 0.0105 | 0.0106 | 0.0105 | **0.0105** |
| **Alkalinity (ppm)** | 118 | 117 | 118 | 115 | 117 | **117** |
| **DO2 (mg / L)** | 5.78 | 5.6 | 5.5 | 5.7 | 5.6 | **5.6** |
| **DCO2 (mg / L)** | 8 | 8.1 | 7.9 | 8 | 7.8 | **7.9** |
| **TDS (ppm)** | 287 | 285 | 286 | 287 | 286 | **286.2** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -)** | + | + | + | + | + | **+** |

**Table: 11. Water parameters of Subhas Sarobar on June & July, 24 (Kolkata, West Bengal)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor**  **Date: June & July, 2024** | | | | | |
| **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 5** | **Average** |
| **Temperature** | 35.4 | 34 | 34.3 | 35.1 | 34.9 | **34.7** |
| **pH** | 7.62 | 6.9 | 7.88 | 8.1 | 7.88 | **7.7** |
| **Salinity (ppt)** | 0.0106 | 0.0106 | 0.0105 | 0.0106 | 0.0105 | **0.0105** |
| **Alkalinity (ppm)** | 119 | 120 | 118 | 120 | 119 | **119.2** |
| **DO2 (mg / L)** | 6.88 | 6.2 | 6.5 | 6.6 | 6.7 | **6.57** |
| **DCO2 (mg / L)** | 09 | 09 | 08 | 09 | 08 | **8.6** |
| **TDS (ppm)** | 334 | 330 | 332 | 334 | 335 | **333** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | 0 | 0 | 0 | **00** |
| **Bacteria (+ / -)** | + | + | + | + | + | **+** |

Physico-chemical parameters of the lake water are shown in Table 5-11. From the table, it was observed that during the study period (9 months), all the physico-chemical parameters except Ammonia, Nitrite, and Nitrate show monthly variations. In the months of May- June temperature of the lake water was maximum (34.70C) and in the months of January- February it showed the minimum value (21.20C). pH value was highest in the month of October (8.42) and least in December (7.6). The value of TDS was max in May- June (333 ppm) and minimum in October (154 ppm). Total alkalinity showed the highest value in October (137 ppm) and least value in March- April (117 ppm). The highest D.O. value was shown in January-February (7.1 mg/L) and the least in November (5.82 mg/L). Dissolve CO2 value was highest in December (8.8 mg/L) and least in March- April (7.9 mg/L). The total hardness value almost remained the same throughout the year.

**Table: 12. Comparative study of water parameters of Subhas Sarobar among Pre-Monsson, Monsoon, and Post Monsoon)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Water Parameters** | **Visit Subhas Sarobor (Pre-Monsson, Monsoon and Post Monsoon)** | | |
| **Pre-Monsson** | **Monsoon** | **Post Monsoon** |
| **Temperature** | 28.9 | 24.7 | **33.34** |
| **pH** | 7.8 | 7.7 | **8.42** |
| **Salinity (ppt)** | 0.0105 | 0.0105 | **0.0105** |
| **Alkalinity (ppm)** | 117 | 119.2 | **137.4** |
| **DO2 (mg / L)** | 5.6 | 6.57 | **5.88** |
| **DCO2 (mg / L)** | 7.9 | 8.6 | **8.6** |
| **TDS (ppm)** | 286.2 | 333 | **154.6** |
| **Ammonia (mg / L)** | 0.5 | 0.5 | **0.5** |
| **Nitrite (mg/L)** | 0.5 | 0.5 | **0.5** |
| **Nitrate (mg / L)** | 0 | 0 | **0** |
| **Bacteria (+ / -)** | +++ | +++++ | **+** |

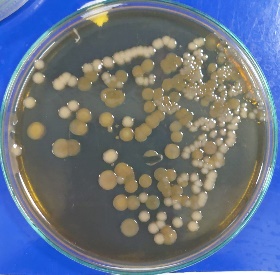
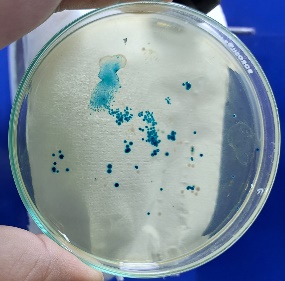
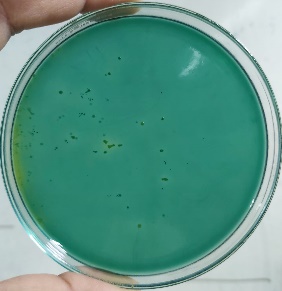
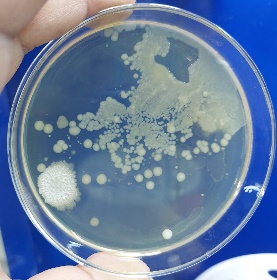
Dissolved oxygen, TDS showed maximum values in monsoon season, whereas pH, alkalinity shows higher values in post monsoon. Ammonia, Nitrite and nitrate remain unchanged throughout the season.

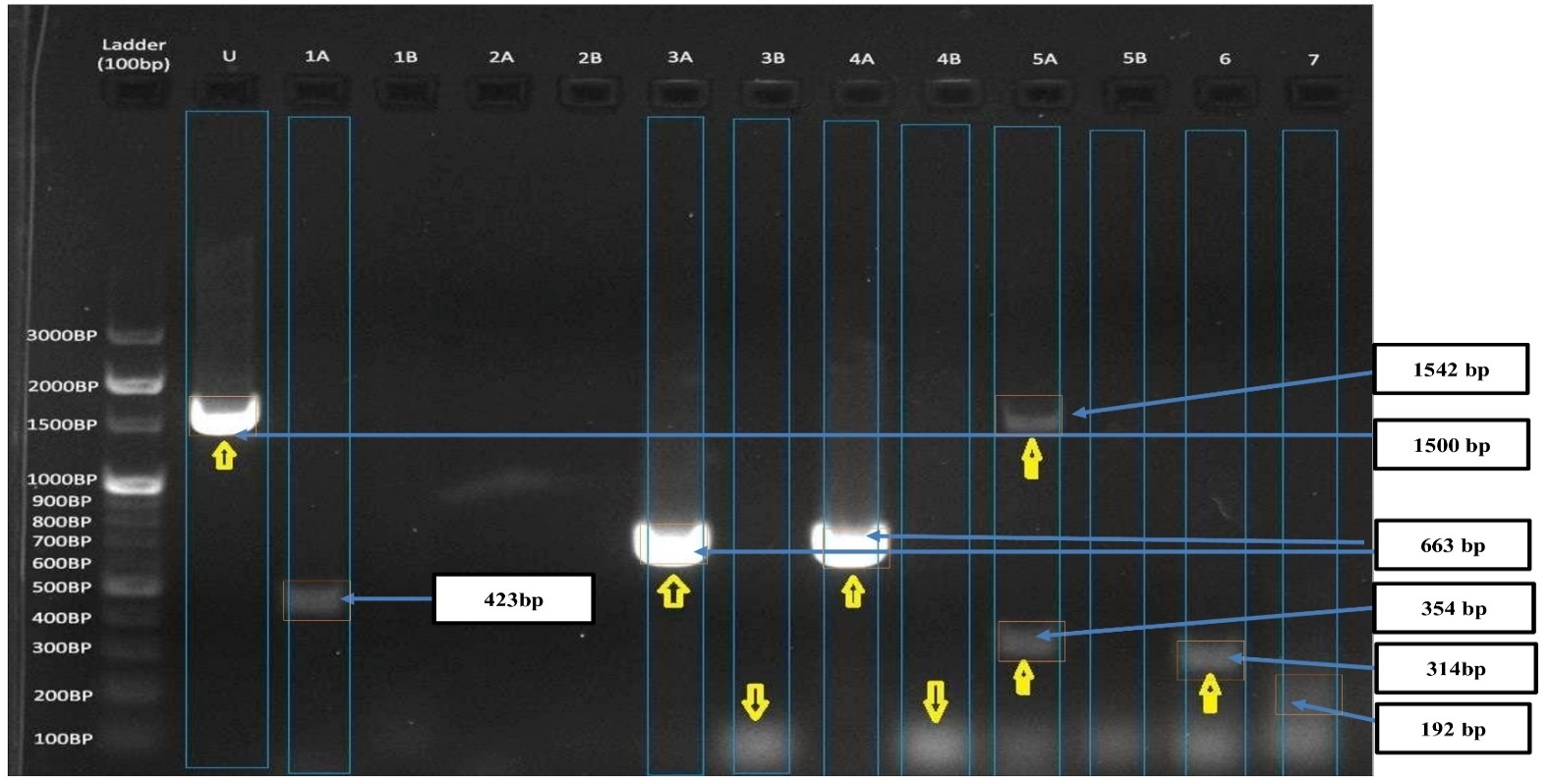
**3.2. Bacterial Diversity in lake water:**

The present study primarily revealed that bacterial populations like *Aeromonas* sp., *E. coli*, *Shigella* sp., *Pseudomonas* sp., *Salmonella* sp., and *Vibrio* sp. are present in the lake water according to kit colour (green to pink to black) (Figure:4; Table:13). Foul water quality, high organic load, contaminated feed, and unhygienic conditions are some of the predisposing factors for an may cause an outbreak of diseases caused by *E. coli*, *Shigella* sp., *Pseudomonas* sp., *Salmonella* sp., *Citrobacter* sp. in local people.

**Table:13.** Colour detection kit for bacteria

|  |  |  |
| --- | --- | --- |
| **Sl. no.** | **Colour** | **Detection of bacteria** |
| 1. | Light green | Control |
| 2. | Deep green | *E. coli* |
| 3. | Blackish green | *Shigella* sp. |
| 4. | Pinkish red | *Vibrio* sp. |
| 5. | Light pink | *Enterobacter* sp. |
| 6. | Black | *Salmonella* sp. |



**Fig: 4.** Water sample culture in different agar

**Figure 5.** Enteropathogenic bacteria [Universal Bacteria-1600, *Vibrio* sp. - 663 bp, *Enterobacter* sp.-1542 bp, *Salmonella* sp. – 314bp, *Shigella* sp. – 423bp, *Yersinia* sp. – 354bp)] were identified in freshwater Subhas Sarobar Lake in WB. The lane at the leftmost indicates the DNA ladder (100 bp).

PCR is a highly sensitive and powerful molecular technique frequently used for the detection of bacteria by targeting specific gene-specific primers. Through PCR assay-based identification, we completed a comprehensive investigation of previously identified bacteria-positive samples from Subhas Sarobar in Kolkata (Choudhury *et al.*, 2024; Figure 4).

When we screened these water samples against universal bacterial primers (27F and 1492R) targeting bacterial rDNA genes, from the results we observed the presence of bacteria primer-specific PCR amplified band corresponding U lane. which indicates the ubiquitous presence of bacterial populations in these regions (Figure 4). Further, we tried to investigate if the enteropathogenic bacterial-specific species causing the disease were present or not. Notably, in the lakes we detected all prominent PCR amplified bands corresponding to and indicating the presence of enteropathogenic bacteria (*Salmonella* sp. – 314 bp; *Yersinia* sp. – 354 bp; *Shigella* sp. – 423 bp; *Vibrio* sp. - 663 bp, *Aeromonas* sp. – 720 bp; *Enterobacter* sp.-1542 bp) (Figure: 5).

**4. Discussion:**

Since the locals utilize the lake for everyday tasks, Subash Sarovar, like most manmade lakes in the nation, is experiencing environmental deterioration as a result of growing bacterial populations, including Shigella sp., Pseudomonas sp., Salmonella sp., Citrobacter sp., and E. coli. Cleaning, swimming, and washing that contaminate and turn the lake's water alkaline. Because more people are living around the lake and visiting, there is also an increase in water pollution. Anthropogenic activities cause the body of water to become eutrophic, meaning it is rich in phosphate and nitrogen. As a result, populations of algae and phytoplankton are growing significantly. In addition, zooplankton and other aquatic life, such as fish and mollusks, are supported in this lake. There is a great deal of decomposition at the lake's bottom because this body of water is home to a vast variety of living things. Consequently, the oxygen needed for aquatic species to survive is reduced. An ideal environment for the growth of harmful bacteria is created by the water body's high BOD value. The health of the locals is at risk because they use this body of water frequently. Human gastrointestinal disorders can be caused by these harmful bacteria. It should be preserved by the government taking action. Additionally, a comprehensive tree planting effort has been initiated by the local government. Garbage dumping into the lake is the primary cause of Sarobar's degradation. Therefore, extensive research is required to identify all harmful microorganisms and regulate the lake water's pollution level. Differential culture of the pathogens and molecular identification of them are also necessary to comprehend the diversity of the bacterial population. Following an analysis of the findings, we are suggesting the following actions to restore the water quality of Subhas Sarobar Lake: refraining from bathing, cleaning clothing and utensils, and disposing of waste products in the water and the surrounding area. Stop fleeing from car washes as well.

**5. Conclusion:**

The molecular detection methods employed in this study revealed a significant shift in bacterial communities across different seasons, with a higher abundance of potentially pathogenic bacteria during the monsoon season. These findings have important implications for public health, as the lake water is used for various purposes. The presence of pathogenic bacteria, such as *Vibrio* sp., *Enterobacter* sp., *Salmonella* sp., *Shigella* sp., *Yersinia* sp. highlights the need for regular monitoring and management of the lake's water quality to prevent waterborne diseases. Furthermore, this study demonstrates the importance of molecular techniques in detecting and characterizing bacterial communities in freshwater ecosystems, providing valuable insights into the ecology and public health implications of these systems. Future studies should focus on exploring the functional roles of these bacterial communities and developing effective strategies for managing and conserving freshwater lakes in the region.

**6. Disclaimer (Artificial intelligence)**

We hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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