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Physicochemical and Bacteriological Analysis of Local Pond Water of Kamarhati Region, Kolkata

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Abstract:

Surface water is both scarce and threatened due to anthropogenic impact of urbanization, growing population and climate change. Fresh water surface quality mainly of ponds and rivers are vulnerable due to various organic loads, emerging pollutants and water borne pathogens. The presence of suspended particles in water also increases the disease probability as the microbes cling on to those surfaces. Unpredictable weather conditions has heightened the susceptibility towards diseases because this ensures the survival of spore/cysts and inflicts pathogenesis. Non-stop rainfall spreads these pathogens in local water bodies like ponds polluting them and drought conditions concentrate these pathogens in those habitats. Drinking water sources then becomes compromised and results in gastrointestinal diseases like diarrhoea and dysentery which are very fatal for children and immune compromised adults. In this research work, two locality ponds were chosen based on their usage by the people of the surrounding area. Our work highlights monitoring the water quality of these two ponds around Kamarhati locality for coliform group by MPN method. Physico-chemical parameters like temperature, pH, turbidity, Total Suspended Solids (TSS), Total Dissolve Solids (TDS), Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD) were also determined as they have influence over microbial growth. Antibiotic susceptibility test of the isolated coliform strains were also performed to ascertain the growing presence of antibiotic resistance in those public use surface water.

Keywords: *Pond, Coliform, Physiochemical, Microbes, Water Quality.*

1. Introduction

Water is an essential element to life on planet Earth. In our daily life we need water to survive, the human body (approximately 60%) is composed of water. Although the earth's surface (75%) is covered by water, it is known fact that only 1% of that is potable. In India, man-made ponds have been used as an alternate source of drinking water and employed for washing of clothes and bathing purposes by washer men and local people [7,8,10,11,20]. The pond water is also affected due to ritual activities by the people living in the nearby areas. The accumulation of various kinds of pollutants and nutrients through the domestic sewage,

municipal effluents, and agricultural runoff leads to the ponds changing its physico-chemical characteristics [4,12,13,14,21]. People exposed to these type of water bodies possess major health risk due to presence of Coliforms. They enter through contaminated faecal-oral route and cause diseases like cholera, diarrhea, dysentery, typhoid and polio [2,3,5,6]. Safeguard of human health and through water quality monitoring and management is an important criteria for countries with poor socioeconomic limitations. Moreover costly and inadequate disinfection techniques fail to eliminate pathogenic microbes from freshwater resources.

Indicator microbes for microbiological water quality analysis falls under Coliform group of Enterobacteriace family. Usually theses coliform are enteric in nature and are discharged from the body through faeces. When drinking water gets contaminated with sewerage pipes or overflowed through surface run offs, then they enter the systems. They are very stable and survives outside the intestine of host for several days and consist of Total coliforms and faecal coliforms. For example, *E. coli* O157:H7, is responsible for causing water borne diseases with mild symptoms to fatal symptoms [17,18]. Presence of these bacteria in surface water gets associated with the users of those water bodies and with unhygienic WASH systems they become huge problem. Conventional Laboratory techniques comprises Most Probable Number method, a statistical approach to enumerate coliform in sample water [6,15,16]. Membrane filtration method also helps in enumerating coliforms when the water sample to be analysed is huge [15]. With increasing pollution load in surface waters, the gravity of these coliform related disease are prevailing. Hence the occurrence of coliform bacteria indicates evidence that water has been contaminated with sewage, and thus enough presence of pathogenic microbes.

Presence of antibiotic resistance is also increasing and it has been found that *E. coli* has shown resistant towards several antibiotics such as ampicillin, ciprofloxacin, streptomycin and others in surface water [19]. There are mainly two main routes to improve water quality like community management, or improving at the 'Point of Use' (POU). Therefore, monitoring and screening of water quality to prevent waterborne incidents are needed to prevent community outbreaks.

2. Materials and methods

2.1 Collection of water samples

Water samples were collected from two ponds of Kamarhati Municipality. This water samples were collected in the month of November 2024 (dry season). Water samples were collected in one liter sterile bottle kept at 4^0C and then immediately transported to the laboratory where they were analyzed.

2.2 Physicochemical Analysis of Water Samples

The water samples were analyzed for the following physicochemical properties: pH, temperature, biological oxygen demand (BOD), total dissolved solids (TDS), total solids (TS), dissolved oxygen (DO) and total suspended solids (TSS) using the methods of American Public Health Association, APHA (1998) [1]. The procedures are followed in the following manner.

2.2.1 For Dissolved oxygen (DO) analysis

1. Water sample was collected in 100 ml BOD bottle.
2. 2 ml of Winkler's A solution and 2ml of Winkler's B solution was added.
3. Stopper was put immediately to eliminate air bubbles and inverted up and down carefully.
4. Brown precipitate was formed which settled down leaving clear supernatant at top.

5. Conc. sulphuric acid was added drop by drop till precipitate was digestate.
6. The bottle was stoppered and mixed for complete dissolution till yellow colored solution was seen.
7. Then few drops of starch indicator was added and titrated against 0.025N $Na_2S_2O_3$ solution.
8. The reading was noted down when the color changed from blue to colorless.

2.2.2 For Biochemcial oxygen demand (BOD) analysis

1. The water sample was taken in 2 BOD bottles.
2. Another two BOD bottles was filled with distilled water.
3. 1 ml each of phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride solutions was added in all above bottles.
4. Then immediately on 1st day DO of the sample and distilled water was estimated.
5. The bottles were incubated at 20°C for 5 days, in BOD incubator undisturbed.
6. DO contain was determined in the incubated bottles at the end of 5th day by using DO estimation method.

2.2.3 Estimation of Total solids (TS)

1. The collected water sample (10 ml) was poured on the petri plate.
2. The initial dry weight of petri plate was note down.
3. The plate is placed inside the oven and heated at 103-105°C
4. After drying in the oven cool the sample was cooled in room temperature
5. The final dry weight of petri plate was noted down.

2.2.4 Estimation of Total suspended solid (TSS)

1. The initial dry weight of the filter paper was noted down.
2. The sample was filtered using filter paper.
3. The water should drain out completely.
4. The filter paper was removed and keep it in hot air oven to dry.
5. The final dry weight of filter paper was noted down.

2.3 Biochemical characterization

IMViC

Indole test

1. The peptone water tubes were inoculated with the isolated bacterial strain to be tested using sterile loop and another without bacterial sample was kept as control.

2. Both tubes were incubated at 37°C for 24-48 hours.

3. Thereafter 1ml of Kovac's reagent was added to both tubes including the control, shaken and result observed after 10 – 15 minutes.

Methyl Red test

1. The MR tubes were inoculated with isolated bacterial strain to be tested using sterile loop and another tube was kept as control.

2. Both tubes incubated at 37°C for 24-48 hours.

3. Thereafter MR indicator was added to both tubes including control, mixed and colour was observed.

VP test

1. The VP broth were inoculated with isolated bacterial strain to be tested using sterile loop and another tube was kept as control.

2. The tubes were incubated at 37°C for 24-48 hours.

3. Then Barrett's reagent A (3 ml) and Barrett's reagent B (1 ml) was added into both tubes including control.

4. The tubes shaken gently for few seconds and result observed after 15 – 30 minutes.

Citrate test

1. In this test agar slant of Simmons citrate was prepared and inoculated with the test bacteria,

2. Both tubes (control and inoculated) were incubated at 37°C for 24 – 48 hours and result observed.

Lactose fermentation

1. Lactose broth was prepared and inoculated with test bacterial culture.

2. Incubated overnight at 37°C

3. Then methyl red as indictor was added to the broth and result observed as color change.

Catalase test

1. For this test, a drop of freshly inoculated bacterial solution was put on sterile slide and 3% H_2O_2 was added on to the bacteria on slide and mixed.

2. Result observed instantly.

2.4 Bacteriological analysis of water samples

To determine the presence of total coliform bacteria, the Most Probable Number (MPN) was performed. This was carried out in three stages which were the presumptive test, confirmed test and completed test [6]. For further identification of an organism in the coliform group, series of biochemical tests were done like Gram stain, IMViC tests, Catalase tests. The catalase test was done for the revealing the presence of enzyme catalase in the bacteria. Lactose fermentation test was done to check the presence of coliform. This tests also

signifies production of acid and/or gas produced from carbohydrate fermentation. The procedure of MPN test is highlighted below.

Presumptive test: Single and double strength lactose broth was prepared at first. Then double strength broth was taken in 5 test tubes and single strength broth was taken in another 10 test tubes. Sample water is added in each test tube and keep for incubation at 37°C for 48 hrs. After the incubation the result was observed. The obtained result was compared from the MPN chart for qualitative analysis of the sample.

Confirmed test: From the tube showing a positive presumptive test, streak of the inoculum was drawn on EMB plate and incubated the plate at 35°C for 24 hrs.

Completed test: Once growth is confirmed, the culture is streaked on the agar slants and after 24 hour incubation the bacterial single colony was Gram stained from the agar slant. (Coliform are gram negative, non-spore forming bacilli)

2.5 Antibiotic sensitivity test

Antibiotic sensitivity test was done to check the resistant property of bacteria in the water sample. Three different kinds of antibiotics were used such as Azithromycin, Amoxicillin and Linezolid. Initially an antibiotic stock was prepared which was diluted to perform the experiment. 2 ml of sterile LB was prepared in the test tube and 10 µL of overnight growth bacterial culture (isolated from EMB plates) and antibiotic solution was added and mixed well.

One control tube was kept which had no antibiotic solution. The tubes were incubated overnight at 37 ^{0}C and the result was observed the next day.

3. Results and Discussion

Physicochemical properties of water

pH is classed as one of the most important water quality parameters. Measurement of pH relates to the acidity or alkalinity of the water. A sample is considered to be acidic if the pH is below 7.0 and alkaline if the pH is higher than 7.0. As per Irrigation Standards of BIS and FAO, pH range should fall between 6.5 – 8.5 [9]. In our result, the pH values were found in the range 7.7 to 7.86 which satisfies the given standard values. Similar results were observed which revealed the slightly alkaline trend in every ponds [2]. TDS level around 200 mg/L is considered as safe for consumption, but in our result the TDS level was found within 400- 500 mg/L, which indicates high buildup of dissolved substances in the pond water. The causes of the increasing TDS is contributed by minerals and algae-promoting nutrients like phosphate. The parameter is also increased mostly by sewage waste, soaps and detergent which comes through bathing and washing [14]. Dissolved oxygen (DO) value were found in the range of 8.732 -7.8 respectively for the two sample ponds tested which indicates moderate amount of oxygen present in them. Comparatively one pond had better DO parameter than the other since DO ranges 8-9 indicate good water quality and 6.7-8 indicate water body is slightly polluted. Dissolved oxygen levels below about 6 mg/L can begin to have detrimental effects on pond life. Biochemical Oxygen Demand (BOD) depends on temperature, extent of biochemical activities and concentration of organic matter and microbial population such as bacteria and fungi [3]. BOD value was found between 1.532-3.47 mg/L, in the two ponds respectively which indicates the low organic waste. Comparatively Pond A seems to have better water quality than Pond B as evident from the Table 1.

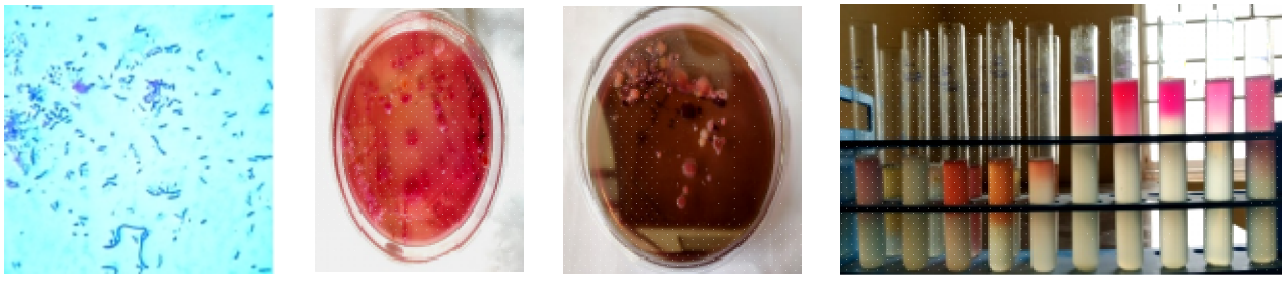


Figure 1. Showing Gram stain, coliform growth on selective media and MPN test

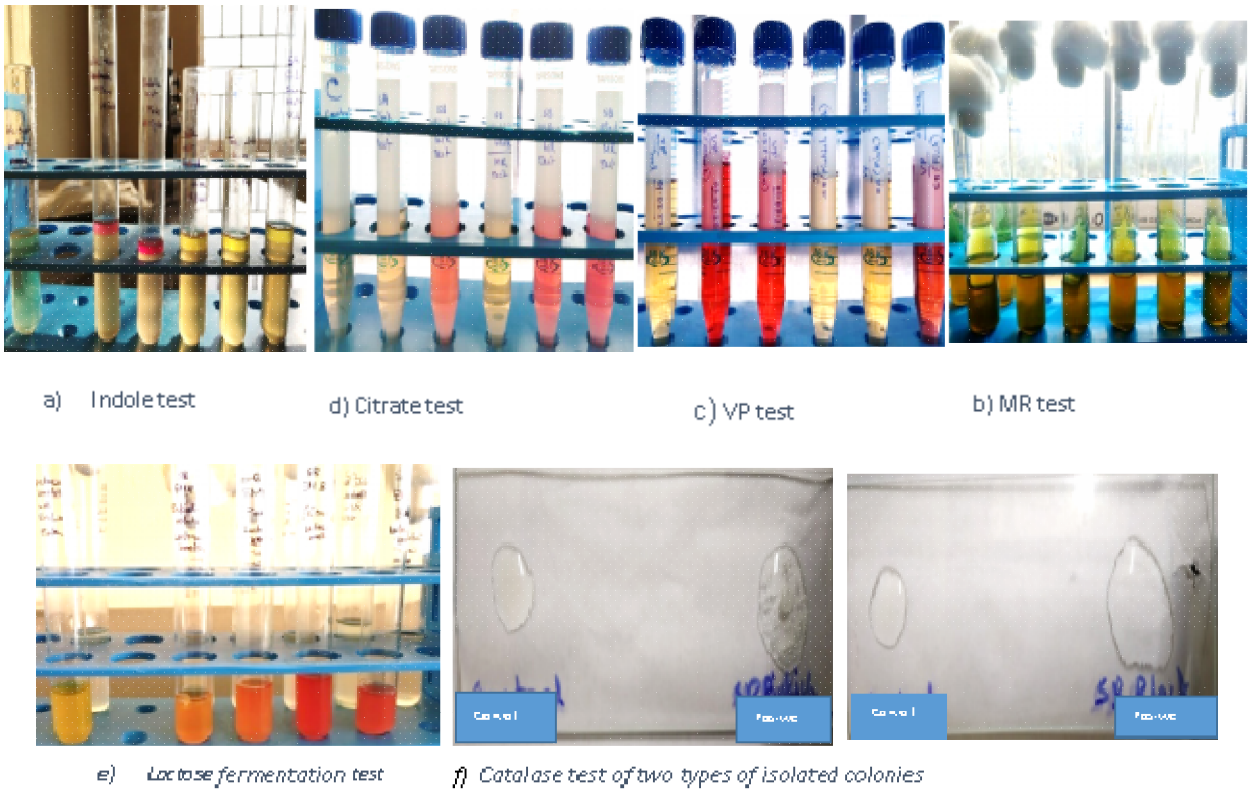


Figure 2. Showing the Biochemical characterization tests of isolated colonies

Table 1. Showing the physio-chemical properties of pond water samples (A and B).

Water Sample from Ponds	TS (mg/l)	TSS (mg/l)	TDS (mg/l)	pH	DO (mg/l)	BOD (mg/l)
Pond A	2400	1900	500	7.77	7.80	3.47
Pond B	2200	1800	400	7.86	8.73	1.532

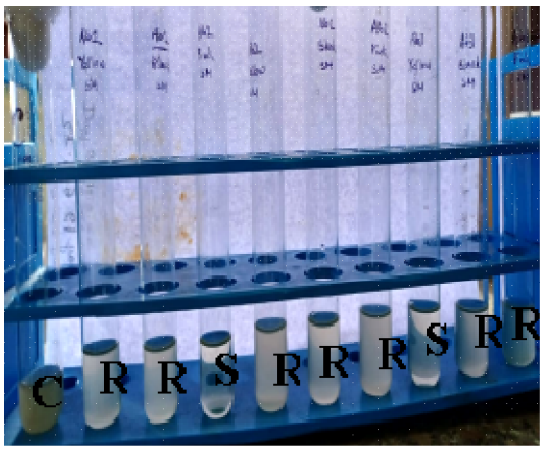
The trophic category of each pond depends on the organic load present in the water sample [3].

Bacteriological examination

Three types of colony black, pink and yellow was observed from the EMB plates. All the colonies were Gram negative, rods with difference in Biochemical characterization parameters (Fig. 1 and Fig. 2). As per WHO guidelines the pond water is not fit for human consumption if it contains more than 50 coliform bacteria per 100 ml of water. Our results from the MPN chart exhibited 170 colonies/100 ml from Pond A and 140 coliform per 100 ml in Pond B which indicates high count of coliform and not safe for people. The reason of high coliform count can be due to the high TDS content of the Pond A compared to Pond B. Presence of high amount of dissolved or suspended particles are also the source of huge contamination where the coliforms get some solid ground to adhere to.

Antibiotic sensitivity test of water

By this test we can confirm about the presence of antibiotic resistant bacteria and it is a great concern for us because people drinking this water directly could develop not only gastrointestinal problems but also ingest antibiotic resistant microbes. The different colonies isolated from the EMB plates of two different pond water displayed high to moderate resistance towards the three type of antibiotics used (Fig. 3). The black colonies which signify *E. coli* were found to be resistant towards all the antibiotics tested in the lab (Table 2). Similar results were obtained by earlier researchers while monitoring the surface waters [19]. Thus people using this water body unknowingly may ingest antibiotic resistant microbes and develop disease which is really of great concern.



Antibiotic	Yellow	Black	Pink
Azithromycin	Resistant	Resistant	Sensitive
Amoxicillin	Resistant	Resistant	Resistant
Linezolid	Sensitive	Resistant	Resistant

Figure 3. Showing Antibiotic sensitivity test in Broth culture from pond water

Table 2. Showing the Antibiotic sensitivity profile of bacteria tested from pond water.

Conclusion

The local water body around Kamarhati municipality is not much safe for people use as because many coliform bacteria may presents in this water so the water quality is deteriorate and also we were found some resistant bacteria from antibiotic sensitivity test which is great concern for us. It is a very alarming situation for those who used this water it may be harmful for their health and also develop gastrointestinal problems. In future more investigations need to be done to find the source of water quality decoration and suitable management approaches need to be developed.

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References:

1. APHA (2005), *Standard Methods for the examination of water and waste water.* AWWA, WPCE, New York, 21st edition.

2. Dixit A, Pandey S, Mehta R, Niyazahmad, Gunjan, Jyotipandey. Study of physico-chemical parameters of different pond water of Bilaspur District, Chhattishgarh, India. Environmental Skeptics and Critics. 2015; 4: 89-95.

3. Mishra S, Singh AL, Tiwary D. Studies of Physico-chemical Status of the Ponds at Varanasi Holy City under Anthropogenic Influences. International Journal of Environmental Research and Development. 2014; 4: 261-268.

4. Rahmanian N, Bt Ali SH, Homayoonfard M, Ali NJ, et al. Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia. Journal of Chemistry 2015; 2015:10.

5. Syeed MMM, Hossain Md S, Karim Md R, Uddin MF, Hasan M, Khan RH. Surface water quality profiling using the water quality index, pollution index and statistical methods: A critical review. Environmental and Sustainability Indicators 2023; 18: 100247.

6. Tasnia A, Baidya S, Mrityunjoy A, Tasmina R. Qualitative analysis of drinking water through the most probable number (MPN) method. Stamford Journal of Microbiology. 2015; 3.

7. Muduli PR, Kumar A, Kanuri VV, Mishra DR, Acharya P, Saha R, Biswas MK, Vidyarthi AK, Sudhakar A. Water quality assessment of the Ganges River during COVID-19 lockdown. International Journal of Environmental Science and Technology. 2021; 18: 1645-1652.

8. Ali S, Singh S, Singh R, Tyagi M, Pandey RP. Influence of multidrug resistance bacteria in river Ganges in the stretch of Rishikesh to Haridwar. Environmental Challenges. 2021; 3: 100068.

9. Joshi DM. Kumar A, Agrawal N. Assessment of the irrigation water quality of river Ganga in Haridwar district. Rasayan J Chem, 2009; 2: 285-292.

10. Paul D. Research on heavy metal pollution of river Ganga: A review. Annals of Agrarian Science. 2017; 15: 278-286.

11. Basu J, Datta S, Bagchi L. A Geographical and Microbiological Assessment of Ganga Water in and Around Dakhshineswar Area, West Bengal.

12. Hur J, Cho J. Prediction of BOD, COD, and total nitrogen concentrations in a typical urban river using a fluorescence excitation-emission matrix with PARAFAC and UV absorption indices. Sensors 2012; 12: 972-986.

13. Gosomji AD, Okooboh MA. Determination of the concentration of dissolved oxygen in water samples from Pankshin town to monitor water pollution. Chem. Materi. Res 2013; 3: 13-17.

14. Islam MR, Sarkar MKI, Afrin T, Rahman SS, Talukder RI, Howlader BK, Khalequ, MA. A study on total dissolved solids and hardness level of drinking mineral water in Bangladesh. Am J Appl Chem. 2016; 4: 164-169.

15. Rompré A, Servais P, Baudart J, de-Roubin MR, Laurent P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J Microbiol Methods. 2002; 49: 31-54.

16. Tambi A, Brighu U, Gupta AB. A sensitive presence/absence test kit for detection of coliforms in drinking water. Water Science & Technology: Water Supply 2016; 16: 1320–1326.

17. Uprety S., Samendra P, Narayanan SP, Dangol B, Maggos M, Celmer A, Shisler J, Amarasiri M, Sano D, Nguyen TH. Microbial assessment of water, sanitation, and hygiene (WaSH) in temporary and permanent settlements two years after Nepal 2015 earthquake, Science of The Total Environment, 2023; 877: 162867.

18. Xu G., Wang T., Wei Y, Zhang Y, Chen J. Fecal coliform distribution and health risk assessment in surface water in an urban-intensive catchment, Journal of Hydrology, 2022, 604:127204.

19. Sério J, Marques AP, Huertas R, Crespo JG, Pereira VJ. Occurrence and Treatment of Antibiotic-Resistant Bacteria Present in Surface Water. Membranes. 2023; 13: 425.

20. Pal D., Maji P. 'Climate Change: Monitoring the Pathogens by Traditional and Latest Trends' in Trends in Environmental Biology, Kripa-Drishti Publications, Chapter 7, pp. 69-75, 2022. ISBN: 978-93-90847-55-6.

21. Maji P., Pal D. 'Photodegradation Processes of Microplastics in Aquatic Medium' in Trends in Environmental Biology. Kripa-Drishti Publications, Chapter 13, pp. 120-126. 2022. ISBN: 978-93-90847-55-6.

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