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Exploring the Anionic Surfactant Concentrations and Biological Contamination in Yamuna River: Identifying Potential Sources and Mitigation Strategies

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Anionic surfactants in the river, particularly downstream in Delhi, have created massive foam-like conditions which may be harmful to aquatic life and humans that come in contact. The present study focussed on the quantification of anionic surfactants, biological contaminants in terms of total bacterial count, total and faecal coliform bacteria and qualitative analysis of pathogenic bacteria from the samples acquired from different sites of Yamuna river. The concentration of anionic surfactants was observed to be between 0.42-3.89 mg L^{-1} at Okhla barrage, which was significantly high as compared to Wazirabad barrage and ITO bridge. The total bacterial count of Okhla barrage was observed more as compared to Wazirabad barrage and ITO Bridge ranging between 9.7×10^6 to 9.1×10^8 CFU/mL. The findings of total coliform bacteria were observed to be consistently high at Wazirabad barrage ranging between 1.3×10^3 to 9.0×10^4 MPN/100 mL. Qualitative analysis of pathogenic bacteria showed the presence of *P. aeruginosa*, *Salmonella* sp., *E. coli* and *S. aureus*. Based on the results obtained in the study, it was inferred that the water quality of Yamuna river at Wazirabad barrage, ITO Bridge and Okhla barrage was poor and thus requires regular monitoring and call for immediate effective mitigation strategies.

Keywords: Total bacterial count, Surfactant pollution, Coliform bacteria, Bioremediation, Pathogens, Remedial strategies.

INTRODUCTION

Ever-increasing urbanization and industrialization is a major reason for making Indian rivers in the vicinity of cities highly susceptible to hazardous pollutants. The situation is extremely severe for one of the largest rivers of India, Yamuna river. Because of several anthropogenic factors and a lack of ecological water flux, it is among the world's most contaminated rivers [1]. For the Delhi-National Capital Region (NCR), river Yamuna is one of the most important sources of raw water. In its 22 km stretch in Delhi, starting from Wazirabad to Okhla Barrage, the extent of pollution in the river makes it unfit for domestic purposes [2,3]. In the last few decades, despite continuous efforts by the local bodies and governments, the pollution in the river has only gone up. Deterioration in the water quality can be attributed to the uncontrolled and unregulated discharge of effluents and dumping of wastes in and around the river [1,4,5]. Pollution in the river is at an alarming situation chiefly in areas downstream of Delhi, which releases and dumps around 58% of the total city waste into the river [6].

Owing to their inherent properties, surfactants are used in detergents as wetting and cleaning agents and thus they are used extensively in households and industries. The coexistence of surfactants in personal care products, pharmaceuticals, agrochemicals, the food industry, *etc.* leads to the excessive, unregulated discharge of surfactants making them a major constituent of wastewater [7] and thus, they enter the surface waters [8]. Amongst all synthetic organic compounds, surfactants are the

top-most produced and consumed chemicals with their demand and consumption rate rising each day [9]. Based on the charge that they carry, surfactants are classified into four categories, among which anionic surfactants constitute approximately 65% of surfactants that are being produced and consumed worldwide [10]. In this study, quantification of anionic surfactants in the Yamuna river water has been carried out.

The natural bacterial diversity present in fresh waterbodies provides them with a self-cleansing ability and allows for the biodegradation of organic matter [11]. The presence of high bacterial loads in the river water can have various implications depending on the specific types of bacteria present. The total bacterial count (TBC) is an indicator of water quality and reflects the level of pollution in a water body.

One of the primary markers for the level of contamination and water quality is the coliform bacterial group. Coliform bacteria are microscopic organisms that are found in the environment, in the faeces of warm-blooded animals and humans. Their presence in water suggests high contamination that ultimately affects organisms utilizing the water as a resource, including humans [10]. Several different types of bacteria fall under total coliform and faecal coliform, both of which are primarily found in faeces. Total coliform is a harmless bacterial group present in soil or vegetation, while faecal coliform is pathogenic and its presence shows faecal contamination [12]. The presence of faecal coliform bacteria like *E. coli* has been known to cause some detrimental health conditions like uremic syndrome, diarrhoea, hemolytic uremic syndrome, hemolytic colitis and newborn meningitis [13].

Investigation of the water quality of a river is considered completed after carefully evaluating and quantifying the nutrients, chemicals and microorganisms, which includes several parameters like the total number of bacterial growths at 22 ºC and 37 ºC [14], total coliforms, faecal coliforms and the presence of pathogenic bacteria. Thus, the current study primarily focuses on the quantification of anionic surfactants and biological contaminants in terms of total bacterial count, total and faecal coliform bacteria and the presence of pathogenic bacteria in the river Yamuna relating them with possible pollution sources and putting forward necessary mitigation strategies.

EXPERIMENTAL

Various bacteriological media and reagents used in the study include nutrient agar (NA), nutrient broth (NB), buffered peptone water, MacConkey broth, brilliant green bile lactose broth (BGBL), eosine methylene blue (EMB) agar, cetramide agar, mannitol salt agar, Baird-Parker agar, Rappaport-Vassiliadis (RV) medium, bismuth sulphide agar, Gram's staining kit, were obtained from Hi-Media, Mumbai, India. Ethanol, sulphanilic acid, isopropyl alcohol (70%), sodium chloride, sodium dodecyl sulphate (SDS) (purity ≥ 98.0%) were of analytical grade and procured from Sigma Laboratories, India. Chloroform (purity ≥ 99.8%) was obtained from Fischer-Scientific, India and methylene blue dye was purchased from Qualikems, India.

Study area and sample collection: Water samples were collected from three different sites from the Yamuna river in the Delhi region. Multiple samples were collected each month from Wazirabad barrage (W), ITO Bridge (I) and Okhla barrage (O), starting from April 2022 to March 2023 (Fig. 1). Samples were collected from the surface using the grab sampling method from a well-mixed zone in triplicates in 500 mL acid-washed containers with air-tight caps, after filtering with Whatman filter paper No. 1 (pore size 11 µm), as prescribed in the standard procedure [15]. The containers were soaked in 10% HNO_3 and rinsed with deionized water 2-3 times. All the samples were immediately carried to the laboratory and stored at 4 ºC until analysis. Collection, preservation and transportation of water samples to the laboratory and analysis were executed as per the American Public Health Association (APHA) standard methods.

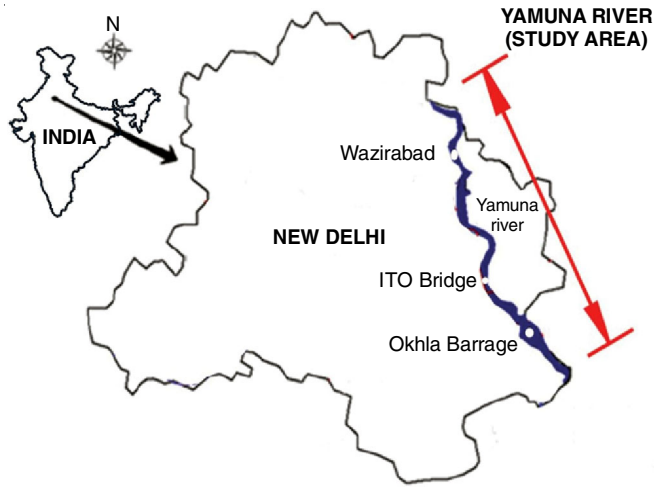

Fig. 1. Geographical representation of the study area highlighting the sampling sites (map not to scale) [Ref. 16]

Analytical method (MBAS assay) for quantification of anionic surfactants: The evaluation of anionic surfactant concentration was done by a standard method known as methylene blue active substances assay (MBAS assay), which uses methylene blue dye and chloroform as solvent [17]. A blue-coloured ionic pair complex is formed between anionic surfactants and methylene blue, which is extracted over chloroform and the absorbance was measured at 652 nm using a UV/visible spectrophotometer (Cary 60, Agilent Technologies).

Assessment of total bacterial population: Quantification of bacteria in each water sample was performed by serially diluting (ratio 1:10; 25 mL sample in 225 mL of 0.85% saline) the sample and following the pour plate method using nutrient agar and incubating the samples at 37 ºC. The total number of colonies on each media was calculated using the following formula:

$$N = \frac{\Sigma C}{(n1 + 0.1n2)d}$$

where N is the sum of colony-forming units in one mL of sample, d stands for the dilution factor from which the primary counts were attained, n1 and n2 correspond to the number of plates considered in the first and second dilution respectively and ΣC indicates the sum of all colonies counted on all the plates [18].

The colonies were observed under a microscope and identical colonies were re-streaked on nutrient agar plates to obtain pure cultures [19].

Estimation of coliform and faecal coliform bacteria: For the estimation of the most probable number (MPN) of total and faecal coliform bacteria in the water samples, standard methods illustrated as per the APHA protocols [20] were followed. This standard procedure comprises three stages.

Enumeration of coliform bacteria in water samples using multiple tube fermentation method: The multiple tube fermentation (MTF) methods, as described in the APHA [19, 20], was carried out for the enumeration of coliform bacteria. A set of 5 test tubes each with 10 mL of double-strength MacConkey broth was taken. Two more similar sets of tubes were taken wherein the same quantity of single-strength MacConkey broth was used. Durham's tube (small tube) was placed inside each tube in an inverted position. Homogenized water samples (10 mL, 1 mL and 0.1 mL) were inoculated in all three sets of test tubes, respectively. All tubes were incubated at 37 ± 1 ºC for 24-48 h for total coliform and at 44.5 ºC for 24-48 h for faecal coliform bacterial enumeration. After incubation, the Durham tube was observed for the production of acid and gas.

Confirmation of coliform bacteria in water samples using brilliant green bile lactose broth: This step is a continuation of the presumptive test. Loopful inoculum from each positive tube was inoculated into 10 mL of BGBL broth. Durham's tube was placed inside each tube in an inverted position. This setup was incubated for 48 h at 37 ± 1 ºC for total coliform and at 44.5 ºC for faecal coliform and observed for gas formation in the Durham tube.

Detection and quantification of faecal coliform bacteria in water samples using eosine methylene blue agar plates: A loopful of inoculum from each positive tube from the confirmative test was streaked onto EMB agar plates for the formation of pure colonies. The plates were incubated at 37 ºC for 24-48 h. Gram staining of the bacteria was performed. Identification of bacterial colonies formed was done as per standard protocols [21]. Formation of green metallic colonies was indicative of faecal coliform bacteria.

The most probable number (MPN) of bacteria was evaluated based on the number of positive tubes in each of the three sets present per 100 mL of water sample using the MPN table [22].

Detection method for *E. coli* in Yamuna water: To detect *E. coli* in the water sample, the membrane filtration method was employed. A 250 mL water sample was passed through a 0.45 µm filter paper, which was then inoculated in MCB. The identification of *E. coli* was confirmed through sub-culturing on EMB agar and MacConkey agar plates. These plates were examined for the presence of pink colonies on MacConkey agar and green metallic sheen colonies on EMB agar plates, which are characteristic of *E. coli*. Further confirmation was carried out through biochemical tests for *E. coli* according to the guidelines outlined in IS: 5887(part-1) [22].

Evaluation: The presence or absence of *E. coli* in the water sample was determined by examining the characteristic colonies on the selective media and conducting morphological and biochemical evaluations. Based on these analyses, the results were recorded as either *E. coli* positive or *E. coli* negative in a 250 mL water sample.

Positive and negative control: Quality control measures were implemented during the experiment by utilizing pure cultures obtained from Microbial Type Culture Collection, Chandigarh. For this experiment, *E. coli* was used as the positive control and *S. aureus* was used as the negative control.

Detection method for *Salmonella* sp. in Yamuna water: To detect the presence of *Salmonella* sp. in water sample, the membrane filtration method was used. A 250 mL water sample was passed through a 0.45 µm filter and the filter paper was then inoculated in buffer peptone water and incubated at 37 ºC for 24 h. After incubation, 0.1 mL of the enriched sample was inoculated in 10 mL of Rappaport-Vassiliadis (RV) medium and then incubated at 42 ºC for 24 h. Subsequently, the sample was subculture on the plates of brilliant green agar and bismuth sulphide agar and the plates were incubated at 37 ºC for 24 h. The colonies were observed for characteristic features such as pink colonies on brilliant green agar and black metallic sheen colonies with H_2S on bismuth sulphide agar plates. Further, confirmation was done by several biochemical and serological tests for *Salmonella* as per the Indian Standard: IS: 5887 (Part3) [23]. To ensure the quality control during the experiment, pure cultures obtained from Microbial Type Culture Collection, Chandigarh, India were used as positive and negative controls, with *Salmonella typhimurium* as positive control and *S. aureus* as the negative control.

Detection method for *P. aeruginosa*: To detect *P. aeruginosa* in Yamuna water sample, a 250 mL sample was passed through a 0.45 µm filter and the filter paper was inoculated in cetrimide broth and incubated at 37 ºC for 48 h. Subsequently, the sample was sub-cultured on cetrimide agar plates and characteristic green colonies were observed. Further confirmation was obtained by gram staining and biochemical tests as per IS: 13428 [24]. The presence or absence of *P. aeruginosa* in the water sample was determined based on the characteristic colonies and biochemical tests. Quality control was maintained by simultaneously running a positive control of *P. aeruginosa* and a negative control of *E. coli* during the experiment.

Detection method for *S. aureus*: To detect *S. aureus*, a 250 mL water sample was passed through a 0.45 µm filter and the filter paper was inoculated in a cooked medium and then incubated at 37 ºC for 24 h. The enriched sample was subcultured on mannitol salt agar and Baird-Parker agar plates. The plates were observed for characteristic colonies such as yellow colonies on mannitol salt agar plates and black colonies on Baird-Parker agar plates. Further confirmation was done by gram staining and biochemical tests as per IS: 5887 (Part-2) [25]. Results were recorded as *S. aureus* positive or negative/ 250 mL of water sample based on characteristic colonies and biochemical tests. During the experiment, the quality control was achieved by running pure cultures of *S. aureus* as positive control and *E. coli* as the negative control simultaneously.

RESULTS AND DISCUSSION

Quantification of anionic surfactants: The concentration of anionic surfactants at three major sites, *viz.*, Wazirabad

barrage, ITO bridge and Okhla barrage, Yamuna river, Delhi, India were carried out using the above-mentioned standard protocols. The results of quantification show a significantly higher concentration of anionic surfactants at Okhla barrage in all the samples collected throughout the year as compared to the other two sites. During the study period, a concentration ranging between 0.42-3.89 mg L^{-1} was found at Okhla barrage, with a yearly average concentration of 2.12 mg L^{-1}.

At Wazirabad barrage and ITO bridge, the anionic surfactant concentration throughout the study period was found to be far less than Okhla barrage. At Wazirabad barrage, the concentration ranged between 0.008-0.65 mg L^{-1}, while at ITO bridge, the concentration of anionic surfactants was found to be in the range of 0.031-0.68 mg L^{-1}. The study shows a substantial reduction in the surfactant concentration at all sampling sites in the monsoon months due to the excess flow of water in river. However, no significant monthwise variation in the concentration of surfactants could be seen at any of the sites during the study period, with a yearly average of 0.33 and 0.38 mg L^{-1}, respectively. Irregular spikes and dips in the surfactant concentration at Wazirabad barrage and ITO bridge have been observed with no sample going above the concentration of 1 mg L^{-1}. Fig. 2 shows the month-wise comparative data of anionic surfactant concentration at Wazirabad barrage, ITO Bridge and Okhla barrage.

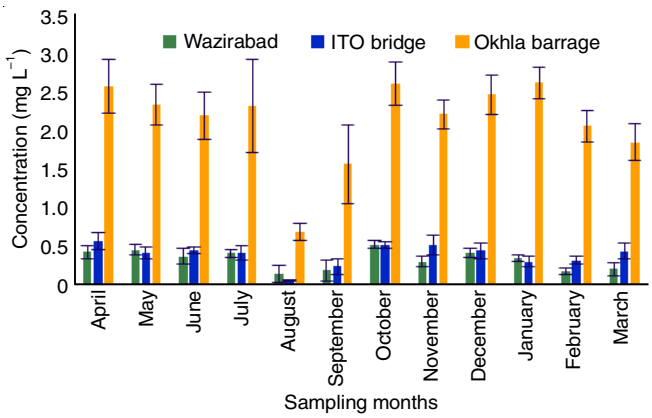

Fig. 2. Comparative analysis of the concentration of anionic surfactants at Wazirabad, ITO Bridge and Okhla Barrage

Assessment of total bacterial count in Yamuna river water: Tables 1-3 show the results of the total bacterial count in the Yamuna river at different locations namely Wazirabad barrage, ITO bridge and Okhla barrage region over a period of 12 months. The total bacterial count (TBC) was measured in colony forming units per mL of water (CFU/mL). In Wazirabad barrage, the results (Table-1) show that the TBC varied greatly over the year, ranging from 1.9×10^6 CFU/mL in April to 9.4×10^8 CFU/mL in W9 in June month. In general, the TBC was higher in the months of June and March, with values exceeding 1×10^7 CFU/mL in most cases. However, the TBC was relatively lower in the months of July, August, September, November, December and January with values generally below 1×10^7 CFU/mL. The lowest TBC was recorded in W4 and W16 in April and July, while the highest TBC was recorded in W9 in June.

In ITO bridge region of Yamuna over the period of eleven months (April to February) indicates a fluctuation in the bacterial population (Table-2). The TBC ranged from 2.3×10^6 CFU/mL in August (sample I16) to 5.9×10^8 CFU/mL in June (sample I9). Overall, the bacterial count was observed to be lower in the winter months (December and January) as compared to the summer months (May and June). The TBC was found to show a decreasing trend in December and January and then increased from February onwards.

Table-3 shows the results of the TBC in CFU/mL for the Okhla barrage region of Yamuna river for a duration of one year. The TBC values range from 9.7×10^6 CFU/mL in December (sample O35) to 9.1×10^8 CFU/mL in March (sample O48). The highest TBC values are observed in the summer months. The TBC data collected from April to March shows that the bacterial count varied greatly across the different locations and months.

Enumeration of total and faecal coliform bacteria

Assessment of total coliform bacteria: Tables 4 and 5 show the level of total and faecal coliform bacteria in the three sampling areas (Wazirabad, ITO Bridge and Okhla Barrage) for the months of April 2022 to March 2023. The data was measured in MPN/100 mL. The level of total coliform bacteria varies greatly among the three sampling areas. At Wazirabad barrage, the level of total coliform bacteria is consistently high throughout the period of April to March, with values ranging from 1.3×10^4 to 9.0×10^4 MPN/100 mL.

At ITO bridge, the level of total coliform bacteria was found to be in the range of 2.8×10^3 MPN/100 mL in November (I29) to 8.0×10^4 MPN/100 mL in April (I4). The results varied significantly over the sampling period with no generalized trend being observed. At Okhla barrage region, the level of total coliform bacteria is also highly variable throughout the period of April to March, with values ranging from 0.22×10^3 – 7.0×10^3 MPN/100 mL (from December to March) to as high as 7.0×10^3 MPN/100 mL (in April).

In general, the levels of total coliform bacteria were highest in April and May 2022 and showed a decreasing trend in the following months. However, there were some exceptions to this trend. In terms of specific locations, it was observed that the levels of total coliform bacteria were consistently highest in Wazirabad barrage throughout the monitoring period, followed by ITO bridge and Okhla barrage. However, it is important to note that the levels of total coliform bacteria in all three locations exceeded the recommended limit for safe drinking water (0 MPN/100 mL) throughout the monitoring period. Therefore, based on the data provided, it can be concluded that the quality of water in Wazirabad barrage, ITO bridge and Okhla barrage was poor and did not meet the recommended standards [26].

Assessment of faecal coliform bacteria: Faecal coliform bacteria levels vary significantly across the three sampling locations (Wazirabad barrage, ITO bridge and Okhla barrage) as well as across different months. For instance, in April, Wazirabad barrage had the highest levels of faecal coliform bacteria (8×10^4 MPN/100 mL), while Okhla barrage had the lowest levels (110 MPN/100 mL). The highest levels at Okhla

TABLE-1
QUANTIFICATION OF TOTAL BACTERIAL COUNT AND QUALITATIVE ESTIMATION OF PATHOGENIC BACTERIA IN WAZIRABAD REGION OF YAMUNA RIVER

Months	Location code	TBC (CFU/mL)	*P. aeroginosa*	*Salmonella* sp.	*E. coli*	*S. aureus*
April	W1	7.3×10^6	Positive	Positive	Positive	Positive
	W2	3.8×10^6	Positive	Positive	Positive	Positive
	W3	8.6×10^7	Positive	Positive	Positive	Positive
	W4	1.9×10^6	Positive	Positive	Positive	Positive
May	W5	5.3×10^7	Positive	Positive	Positive	Positive
	W6	6.8×10^6	Positive	Positive	Positive	Positive
	W7	3.6×10^7	Positive	Positive	Positive	Positive
	W8	8.1×10^6	Positive	Positive	Positive	Positive
June	W9	9.4×10^8	Positive	Positive	Positive	Positive
	W10	4.1×10^6	Positive	Positive	Positive	Positive
	W11	5.8×10^7	Positive	Positive	Positive	Positive
	W12	4.7×10^7	Positive	Positive	Positive	Positive
July	W13	3.1×10^6	Positive	Positive	Positive	Positive
	W14	2.5×10^6	Negative	Negative	Positive	Negative
	W15	2.4×10^6	Negative	Negative	Positive	Negative
	W16	1.9×10^6	Negative	Negative	Positive	Negative
August	W17	2.2×10^7	Positive	Positive	Positive	Positive
	W18	5.8×10^6	Positive	Positive	Positive	Positive
	W19	8.2×10^6	Positive	Positive	Positive	Positive
	W20	3.9×10^6	Negative	Negative	Positive	Negative
September	W21	4.8×10^6	Negative	Negative	Positive	Negative
	W22	7.3×10^6	Positive	Positive	Positive	Positive
	W23	1.9×10^8	Positive	Positive	Positive	Positive
	W24	6.2×10^6	Positive	Positive	Positive	Positive
October	W25	5.2×10^7	Positive	Positive	Positive	Positive
	W26	6.8×10^6	Positive	Positive	Positive	Positive
	W27	8.4×10^7	Positive	Positive	Positive	Positive
	W28	7.2×10^7	Positive	Positive	Positive	Positive
November	W29	9.3×10^7	Positive	Positive	Positive	Positive
	W30	5.9×10^6	Positive	Positive	Positive	Positive
	W31	2.4×10^6	Negative	Negative	Positive	Negative
	W32	7.2×10^7	Positive	Positive	Positive	Positive
December	W33	9.6×10^6	Positive	Positive	Positive	Positive
	W34	4.6×10^6	Positive	Positive	Positive	Positive
	W35	3.4×10^6	Positive	Positive	Positive	Positive
	W36	5.6×10^6	Positive	Positive	Positive	Positive
January	W37	6.1×10^6	Positive	Positive	Positive	Positive
	W38	8.6×10^7	Positive	Positive	Positive	Positive
	W39	4.9×10^7	Positive	Positive	Positive	Positive
	W40	6.5×10^6	Positive	Positive	Positive	Positive
February	W41	2.8×10^7	Positive	Positive	Positive	Positive
	W42	6.9×10^6	Positive	Positive	Positive	Positive
	W43	5.8×10^7	Positive	Positive	Positive	Positive
	W44	7.4×10^6	Positive	Positive	Positive	Positive
March	W45	8.2×10^7	Positive	Positive	Positive	Positive
	W46	5.6×10^7	Positive	Positive	Positive	Positive
	W47	4.7×10^7	Positive	Positive	Positive	Positive
	W48	1.8×10^8	Positive	Positive	Positive	Positive

barrage was reported in the month of February (2.3×10^3 MPN/100 mL), while Wazirabad barrage had the lowest levels (300 MPN/100 mL) in the month of December. Over the course of the year, faecal coliform bacteria levels generally decreased, with the lowest levels being observed in December and January. This trend may be due to seasonal factors, such as changes in temperature or precipitation.

Despite the overall decreasing trend, there were some spikes in faecal coliform bacteria levels, such as in May at Wazirabad barrage and ITO bridge and in October at Okhla barrage. These spikes may be due to factors such as heavy rainfall, sewage overflows or other forms of contamination. However, the faecal coliform bacteria levels observed (Table-4) exceed the maximum permissible limits for safe bathing water in India (500 MPN/100 mL), according to the Central Pollution Control Board (CPCB), New Delhi, India. This suggests that the water in these areas may not be safe for recreational activities such as swimming and also for other domestic uses.

The results show the levels of faecal coliform bacteria (MPN/100 mL) at three different sampling points (Wazirabad

TABLE-2
QUANTIFICATION OF TOTAL BACTERIAL COUNT AND QUALITATIVE ESTIMATION OF PATHOGENIC BACTERIA IN ITO BRIDGE REGION OF YAMUNA RIVER

Months	Location code	TBC (CFU/mL)	*P. aeroginosa*	*Salmonella* sp.	*E. coli*	*S. aureus*
April	I1	6.8×10^6	Positive	Positive	Positive	Positive
	I2	5.1×10^7	Positive	Positive	Positive	Positive
	I3	9.2×10^6	Positive	Positive	Positive	Positive
	I4	3.9×10^7	Positive	Positive	Positive	Positive
May	I5	8.3×10^7	Positive	Positive	Positive	Positive
	I6	6.1×10^7	Positive	Positive	Positive	Positive
	I7	5.7×10^7	Positive	Positive	Positive	Positive
	I8	3.9×10^7	Positive	Positive	Positive	Positive
June	I9	6.8×10^8	Positive	Positive	Positive	Positive
	I10	3.8×10^7	Positive	Positive	Positive	Positive
	I11	9.4×10^6	Positive	Positive	Positive	Positive
	I12	1.1×10^7	Positive	Positive	Positive	Positive
July	I13	4.3×10^6	Negative	Negative	Positive	Negative
	I14	3.9×10^6	Negative	Negative	Positive	Negative
	I15	5.1×10^7	Positive	Positive	Positive	Positive
	I16	2.4×10^6	Negative	Negative	Positive	Negative
August	I17	1.6×10^7	Positive	Positive	Positive	Positive
	I18	2.3×10^6	Negative	Negative	Positive	Negative
	I19	2.6×10^7	Positive	Positive	Positive	Positive
	I20	1.5×10^7	Positive	Positive	Positive	Positive
September	I21	6.3×10^7	Positive	Positive	Positive	Positive
	I22	9.0×10^6	Positive	Positive	Positive	Positive
	I23	4.6×10^7	Positive	Positive	Positive	Positive
	I24	5.9×10^7	Positive	Positive	Positive	Positive
October	I25	6.6×10^6	Positive	Positive	Positive	Positive
	I26	3.7×10^7	Positive	Positive	Positive	Positive
	I27	7.6×10^7	Positive	Positive	Positive	Positive
	I28	8.4×10^6	Positive	Positive	Positive	Positive
November	I29	6.3×10^7	Positive	Positive	Positive	Positive
	I30	3.7×10^7	Positive	Positive	Positive	Positive
	I31	6.3×10^7	Positive	Positive	Positive	Positive
	I32	8.8×10^6	Positive	Positive	Positive	Positive
December	I33	6.5×10^6	Positive	Positive	Positive	Positive
	I34	3.9×10^6	Positive	Positive	Positive	Positive
	I35	4.2×10^6	Negative	Negative	Positive	Negative
	I36	7.0×10^6	Positive	Positive	Positive	Positive
January	I37	7.3×10^6	Positive	Positive	Positive	Positive
	I38	9.5×10^6	Positive	Positive	Positive	Positive
	I39	2.8×10^7	Positive	Positive	Positive	Positive
	I40	5.5×10^6	Negative	Negative	Positive	Negative
February	I41	1.7×10^7	Positive	Positive	Positive	Positive
	I42	3.8×10^7	Positive	Positive	Positive	Positive
	I43	6.2×10^6	Positive	Positive	Positive	Positive
	I44	4.4×10^7	Positive	Positive	Positive	Positive
March	I45	2.9×10^7	Positive	Positive	Positive	Positive
	I46	3.8×10^7	Positive	Positive	Positive	Positive
	I47	5.9×10^8	Positive	Positive	Positive	Positive
	I48	3.2×10^8	Positive	Positive	Positive	Positive

barrage, ITO bridge and Okhla barrage) in the river Yamuna over a period of nine months (April to December). The data suggest that the levels of faecal coliform bacteria in the Yamuna river are consistently high throughout the sampling period, with occasional fluctuations. Okhla barrage appears to have the highest levels of contamination, followed by Wazirabad barrage and ITO bridge.

Qualitative estimation of pathogenic bacteria: Table-1 shows the qualitative estimation of pathogenic bacteria in the Wazirabad barrage of Yamuna river over a period of 12 months, from April to March. It can be observed that all locations showed positive results for *P. aeruginosa*, *Salmonella* sp., *E. coli* and *S. aureus* in the months of April 2022-March 2023, except for W14, W15, W20, W21, W31, which showed negative results for *Salmonella* sp. and *S. aureus*.

In ITO bridge area, Table-2 shows that, in general, all water samples showed the positive results for all four bacteria during several months. However, there were a few samples (I13, I14, I18, I35, I40) that showed negative results for one or more of the bacteria during certain months. In particular, *Salmonella*

TABLE-3
QUANTIFICATION OF TOTAL BACTERIAL COUNT AND QUALITATIVE ESTIMATION OF PATHOGENIC BACTERIA IN OKHLA BARRAGE REGION OF YAMUNA RIVER

Months	Location code	TBC (CFU/mL)	*P. aeroginosa*	*Salmonella* sp.	*E. coli*	*S. aureus*
April	O1	7.3×10^8	Positive	Positive	Positive	Positive
	O2	8.5×10^7	Positive	Positive	Positive	Positive
	O3	3.9×10^7	Positive	Positive	Positive	Positive
	O4	1.6×10^8	Positive	Positive	Positive	Positive
May	O5	2.4×10^8	Positive	Positive	Positive	Positive
	O6	5.8×10^7	Positive	Positive	Positive	Positive
	O7	6.9×10^7	Positive	Positive	Positive	Positive
	O8	9.4×10^7	Positive	Positive	Positive	Positive
June	O9	5.6×10^8	Positive	Positive	Positive	Positive
	O10	6.7×10^8	Positive	Positive	Positive	Positive
	O11	3.8×10^8	Positive	Positive	Positive	Positive
	O12	6.8×10^7	Positive	Positive	Positive	Positive
July	O13	7.9×10^7	Positive	Positive	Positive	Positive
	O14	8.2×10^7	Positive	Positive	Positive	Positive
	O15	2.9×10^7	Positive	Positive	Positive	Positive
	O16	6.9×10^7	Positive	Positive	Positive	Positive
August	O17	8.7×10^7	Positive	Positive	Positive	Positive
	O18	1.8×10^7	Negative	Negative	Positive	Negative
	O19	6.7×10^7	Positive	Positive	Positive	Positive
	O20	2.9×10^7	Negative	Negative	Positive	Negative
September	O21	5.2×10^7	Positive	Positive	Positive	Positive
	O22	1.2×10^8	Positive	Positive	Positive	Positive
	O23	7.1×10^7	Positive	Positive	Positive	Positive
	O24	6.8×10^7	Positive	Positive	Positive	Positive
October	O25	8.2×10^7	Positive	Positive	Positive	Positive
	O26	9.1×10^7	Positive	Positive	Positive	Positive
	O27	6.3×10^8	Positive	Positive	Positive	Positive
	O28	4.7×10^7	Positive	Positive	Positive	Positive
November	O29	8.1×10^7	Positive	Positive	Positive	Positive
	O30	6.8×10^7	Positive	Positive	Positive	Positive
	O31	9.3×10^7	Positive	Positive	Positive	Positive
	O32	2.9×10^7	Positive	Positive	Positive	Positive
December	O33	8.4×10^7	Positive	Positive	Positive	Positive
	O34	6.1×10^7	Positive	Positive	Positive	Positive
	O35	9.7×10^6	Positive	Positive	Positive	Positive
	O36	2.5×10^7	Positive	Positive	Positive	Positive
January	O37	6.9×10^7	Positive	Positive	Positive	Positive
	O38	7.1×10^7	Positive	Positive	Positive	Positive
	O39	8.9×10^7	Positive	Positive	Positive	Positive
	O40	3.8×10^8	Positive	Positive	Positive	Positive
February	O41	2.9×10^8	Positive	Positive	Positive	Positive
	O42	5.9×10^8	Positive	Positive	Positive	Positive
	O43	7.1×10^7	Positive	Positive	Positive	Positive
	O44	6.4×10^8	Positive	Positive	Positive	Positive
March	O45	3.1×10^8	Positive	Positive	Positive	Positive
	O46	7.7×10^8	Positive	Positive	Positive	Positive
	O47	5.6×10^8	Positive	Positive	Positive	Positive
	O48	9.1×10^8	Positive	Positive	Positive	Positive

sp. and *S. aureus* were absent from several locations for some months.

The qualitative estimation of pathogenic bacteria in Okhla barrage region of Yamuna river indicates the presence of four types of bacteria: *P. aeruginosa*, *Salmonella* sp., *E. coli* and *S. aureus*. As seen in Table-3, in April, all four locations (O1, O2, O3 and O4) tested positive for all four types of bacteria. This trend continued in May, June and July, with all locations testing positive for all four types of bacteria. In August, there was a change in trend with two locations (O18 and O20) testing negative for *Salmonella* sp. and *S. aureus*. However, all other locations continued to test positive for all four types of bacteria. From the months of September 2022 to March 2023, all the samples were found to be positive for all four types of bacteria, with no negative results. The results suggests a consistent presence of pathogenic bacteria in the Okhla barrage region of Yamuna river throughout the studied period, with no significant seasonal or temporal variation.

TABLE-4
EVALUATION OF TOTAL COLIFORM BACTERIA AT THE THREE SAMPLING SITES FOR A STUDY PERIOD OF ONE YEAR

Sampling months	Total coliform bacteria (MPN/100 mL)					
	Wazirabad (W)		ITO bridge (I)		Okhla barrage (O)	
April	W1	7×10^4	I1	3.4×10^4	O1	500
	W2	5×10^4	I2	5×10^4	O2	1.6×10^3
	W3	7×10^4	I3	5×10^4	O3	700
	W4	8×10^3	I4	8×10^4	O4	500
May	W5	5×10^4	I5	2.1×10^4	O5	700
	W6	9×10^4	I6	9×10^3	O6	500
	W7	5×10^4	I7	2.7×10^4	O7	700
	W8	9×10^3	I8	3.4×10^4	O8	2.6×10^3
June	W9	7×10^3	I9	5×10^4	O9	1.7×10^3
	W10	2.8×10^4	I10	5×10^4	O10	700
	W11	7×10^4	I11	3.4×10^4	O11	500
	W12	5×10^4	I12	5×10^4	O12	700
July	W13	2.6×10^4	I13	2.2×10^4	O13	800
	W14	9×10^3	I14	5×10^4	O14	700
	W15	3.5×10^4	I15	7×10^4	O15	350
	W16	3×10^4	I16	5×10^4	O16	500
August	W17	5×10^3	I17	5×10^3	O17	800
	W18	1.3×10^3	I18	3.4×10^4	O18	700
	W19	5×10^3	I19	9×10^3	O19	500
	W20	7×10^4	I20	2.7×10^4	O20	2.7×10^3
September	W21	2.2×10^4	I21	5×10^4	O21	500
	W22	9×10^3	I22	7×10^4	O22	500
	W23	2.6×10^4	I23	1.7×10^4	O23	800
	W24	7×10^4	I24	3.4×10^4	O24	1.6×10^3
October	W25	3.5×10^4	I25	8×10^3	O25	700
	W26	7×10^4	I26	3.3×10^4	O26	900
	W27	8×10^3	I27	2.3×10^4	O27	500
	W28	5×10^4	I28	7×10^4	O28	700
November	W29	1.6×10^4	I29	2.8×10^3	O29	700
	W30	3.4×10^4	I30	5×10^4	O30	270
	W31	7×10^4	I31	3.3×10^4	O31	350
	W32	3.5×10^4	I32	7×10^4	O32	2.8×10^3
December	W33	5×10^4	I33	1.6×10^4	O33	220
	W34	2.6×10^4	I34	8×10^3	O34	2.8×10^3
	W35	2.7×10^3	I35	7×10^3	O35	3.5×10^3
	W36	8×10^3	I36	9×10^3	O36	350
January	W37	9×10^3	I37	1.7×10^4	O37	2.8×10^3
	W38	5×10^4	I38	2.8×10^4	O38	5×10^3
	W39	5×10^4	I39	8×10^3	O39	500
	W40	1.7×10^3	I40	5×10^4	O40	700
February	W41	2.8×10^4	I41	3.5×10^4	O41	900
	W42	3.4×10^4	I42	5×10^4	O42	7×10^3
	W43	7×10^4	I43	3.5×10^3	O43	240
	W44	5×10^4	I44	8×10^3	O44	700
March	W45	8×10^3	I45	7×10^4	O45	900
	W46	2.6×10^3	I46	3.4×10^4	O46	1.6×10^3
	W47	7×10^4	I47	3.4×10^4	O47	700
	W48	2.6×10^4	I48	7×10^4	O48	1.6×10^3

Delhi dumps 58% of its waste into the Yamuna river. Most of the pollution in the river comes from Wazirabad barrage (from where it enters Delhi) and downstream [6,27]. The wastewater from mostly domestic activities enters the river and accounts for the presence of high detergents and phosphate compounds. Anionic surfactants are the most common ingredient of detergents and cause significant deleterious effects to both biotic and abiotic components of the ecosystem [28]. Foaming of Yamuna river at Okhla barrage can be attributed to high surfactant pollution which is evident by present results. The concentration of anionic surfactants found at Okhla barrage is far above the desirable and permissible limits as per the standards. A desirable limit of 0.2 mg L^{-1} and a maximum permissible limit of 1.0 mg L^{-1} of surfactants in water have been adopted in India [29]. High surfactant concentrations at Okhla barrage can be linked to the washing of clothes near Dhobi ghat (a proximity to Okhla barrage), bathing near the river shores and discharge of effluents and multiple drains and

TABLE-5
EVALUATION OF TOTAL COLIFORM BACTERIA AT THE THREE SAMPLING SITES FOR A STUDY PERIOD OF ONE YEAR

Sampling months	Total coliform bacteria (MPN/100 mL)					
	Wazirabad (W)		ITO bridge (I)		Okhla barrage (O)	
April	W1	7×10^3	I1	2.7×10^3	O1	110
	W2	5×10^3	I2	5×10^3	O2	700
	W3	9×10^3	I3	2.1×10^4	O3	130
	W4	1.6×10^3	I4	8×10^3	O4	140
May	W5	2.7×10^3	I5	2.3×10^3	O5	130
	W6	1.7×10^3	I6	2.1×10^3	O6	110
	W7	2.1×10^4	I7	5×10^3	O7	220
	W8	1.6×10^3	I8	7×10^3	O8	900
June	W9	3.5×10^3	I9	5×10^3	O9	500
	W10	8×10^4	I10	5×10^3	O10	170
	W11	9×10^3	I11	2.6×10^3	O11	170
	W12	5×10^3	I12	1.3×10^4	O12	220
July	W13	1.1×10^4	I13	3.5×10^3	O13	130
	W14	1.3×10^3	I14	2600	O14	330
	W15	5×10^3	I15	900	O15	210
	W16	1.6×10^3	I16	1.3×10^3	O16	110
August	W17	900	I17	1.7×10^3	O17	140
	W18	800	I18	5×10^3	O18	110
	W19	7×10^4	I19	3.4×10^4	O19	270
	W20	7×10^3	I20	1.7×10^3	O20	700
September	W21	5×10^3	I21	1.3×10^3	O21	160
	W22	700	I22	8.6×10^3	O22	130
	W23	2.6×10^3	I23	5×10^3	O23	130
	W24	5×10^3	I24	5×10^3	O24	210
October	W25	1.6×10^4	I25	7×10^3	O25	330
	W26	8×10^3	I26	1.4×10^3	O26	350
	W27	1.7×10^3	I27	7×10^3	O27	130
	W28	1.6×10^4	I28	5×10^3	O28	220
November	W29	1.6×10^3	I29	1.4×10^3	O29	140
	W30	5×10^3	I30	8×10^3	O30	110
	W31	8×10^3	I31	2.6×10^3	O31	280
	W32	1.3×10^4	I32	5×10^3	O32	900
December	W33	5×10^3	I33	800	O33	160
	W34	700	I34	2.8×10^3	O34	170
	W35	350	I35	300	O35	340
	W36	500	I36	2.7×10^3	O36	130
January	W37	2.2×10^3	I37	3.5×10^3	O37	330
	W38	1.6×10^4	I38	2.4×10^3	O38	800
	W39	8×10^3	I39	1.6×10^3	O39	110
	W40	1.3×10^3	I40	9×10^3	O40	240
February	W41	5×10^3	I41	2.7×10^3	O41	160
	W42	7×10^3	I42	7×10^3	O42	2.3×10^3
	W43	7×10^4	I43	3.4×10^4	O43	130
	W44	5×10^3	I44	3×10^3	O44	300
March	W45	1.7×10^3	I45	3.4×10^3	O45	260
	W46	300	I46	5×10^4	O46	700
	W47	900	I47	1.6×10^4	O47	110
	W48	3.5×10^3	I48	8×10^3	O48	240

joining of highly polluted Hindon river [30]. It is to be noted that Hindon river, a tributary of river Yamuna, receives non-treated industrial wastes and sewage. Yamuna river at Kalindi Kunj dam is joined by 70% of the water from Hindon from where it enters the sampling site of Okhla barrage.

The results obtained in the current study show significantly higher values of anionic surfactant in Yamuna river as compared to the other existing studies and call for immediate and effective action for its remediation. Several studies have been conducted in the past on bacterial diversity in river Yamuna which strongly suggest significantly high bacterial contamination in the river. A study conducted in the year 2021 shows the maximum total bacterial count obtained was 1.5×10^7 CFU/mL [31]. In present study as well, the total bacterial count ranged between $1.9 \times 10^6 - 9.1 \times 10^8$ CFU/mL, which shows that no improvement in the biological contamination of river has taken place. Considered as faecal indicator bacteria, coliforms are found in the contaminated environments. One of the major sources of conta-

mination in underground and surface water in underdeveloped and developing countries [32], microbes like faecal coliforms burden of the pathogens on the water can be identified. Also, an assessment of other pathogenic bacteria like *S. aureus* and *P. aeruginosa* is performed to understand the level of pathogenicity and identify the presence of disease-causing bacteria in the water samples [33]. As per the studies conducted previously, a high level of contamination of the river with coliform bacteria was found in the Yamuna river, making it unfit for consumption and other domestic purposes. A study conducted by Pali [30] showed high coliform bacteria in the stretch between Wazirabad barrage and Okhla Barrage which is in agreement with the present results. Both total and faecal coliform bacteria were found in high MPNs/100 mL of water samples. However, it is to be observed that both total and coliform bacteria were found to be less in number at Okhla barrage as compared to the other two sites. An important reason for this might be the high concentration of anionic surfactants which possess antimicrobial properties. In another similar study that assessed the coliform bacteria in Yamuna river in the Delhi stretch for the years 2007-2016, a similar trend was observed where total coliform was found between 1.0×10^4 to 9.2×10^8 MPN/100 mL exceeding the desired levels of standards. The authors suggested that such high numbers of coliform bacteria could be the result of high organic matter and also due to the release of untreated wastewater from several drains across Delhi [12]. The presence of faecal coliform bacteria are indicative of the presence of pathogenic bacteria as well. Such pathogenic contamination of the river can be a result of lack of or no connectivity between drains and sewage treatment plants, leading to untreated sewage discharge, faecal contamination, *etc.* [34].

In the current study, the quality of water in Wazirabad barrage, ITO Bridge and Okhla barrage during the monitoring period of one year was evaluated by examining the level of TBC, total and faecal coliform bacteria present in the water samples. From the data attained in the present study, it was observed that the levels of total and faecal coliform bacteria varied throughout the monitoring period. Moreover, the results also suggest that the Yamuna river is heavily contaminated with coliform bacteria, posing a significant public health risk to those who use the river water for drinking, bathing and other purposes. The high levels of contamination may be attributed to untreated sewage and industrial waste being discharged into the river, as well as agricultural runoff and other sources of pollution. A qualitative analysis of pathogenic bacteria in the Yamuna river was carried out, which shows the presence of harmful disease-causing bacteria in the water samples and strongly suggests that no significant improvement in the water quality has taken place over the last few years when compared the present data with the other similar reported works [35-40]. The presence of pathogenic bacteria indicates that the water quality is not suitable for human consumption and other domestic purposes and can pose a risk to human health. Continuous monitoring and proper treatment of wastewater are necessary to ensure the safety of water resources and reduce the risk of waterborne diseases.

Conclusion

Assessment of the levels of biological contamination and anionic surfactants in the Yamuna river across Delhi has been the attempt of this study. The research findings suggest that the Yamuna river is contaminated with anionic surfactants and has excessively high quantities of biological contaminants in terms of total bacterial count, total and faecal coliform bacteria and disease-causing pathogens. The anionic surfactant concentration was found to be higher than the permissible limit at some locations and exceeded the desirable limit at most of the sites during the study period. The excessive foaming of the river at Okhla barrage further supports the presence of surfactant pollution in the river. Regarded as one of the important markers of severe water pollution, the presence of such high range of microbial communities in the river water is a serious cause of concern. The bacteriological parameters investigated in the study were not found in compliance with the WHO standards, highlighting the need for immediate action to address the issue in terms of formulation of mitigation strategies. Unregulated and uncontrolled discharge of industrial and household effluents and dumping of waste including animal carcasses, *etc.* must come under regulation. Such severe contamination of a river, which is a major source of raw water for the population of Delhi has raised serious concerns over human health and ecosystem functioning. The study provides important background data for future remedial studies, particularly the use of native bacterial species for bioremediation technology. This approach can help mitigate the adverse effects of surfactant pollution in the Yamuna river. Overall, the findings underscore the need for effective regulations and monitoring of waste discharge into the river to protect its ecological and human health.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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