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BACTERIAL STATUS AND POLLUTION LEVEL IN THE WATER OF GULSHAN LAKE, DHAKA

Md. Abdul Karim*, Nazneen Akhter and Sirajul Hoque[1]

Laboratory of Microbiology, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

Abstract: Water samples were collected from four selected sites of the Gulshan lake during four different seasons. Four different media were used to study the aerobic heterotrophic, enteric and related bacterial abundance. Aerobic heterotrophic bacterial abundance ranged between 1×10^6 and 9×10^6 cfu/100 ml, while enteric and related bacterial abundance in SS agar, MacConkey agar and Cetrimide agar media ranged between 1×10^3 and 3×10^5 cfu/100 ml, between 1×10^4 and 1×10^7, and between 1×10^0 and 1.4×10^3 cfu/100 ml, respectively. Among 40 bacterial isolates 14 were selected out of which seven were Gram-positive and seven were Gram-negative. Among Gram-positive isolates, six were rod-shaped spore former belonging to *Bacillus* and *Lactobacillus,* while another one was coccoid in shape and under the genus *Planococcus*. Out of seven Gram-negative isolates, two were the members of *Pseudomonas*, remaining five were *Morganella*, *Neisseria*, *Escherichia*, *Aeromonas* and *Enterobacter*. Inorganic nutrients like NH_4^+-N, NO_3^--N, NO_2^--N and PO_4^- values were found to be satisfactory from pollution point of view. The presence of different bacterial isolates indicate that the Gulshan lake water was polluted with chemical and bacterial pollutants.

Key words: Aerobic heterotrophic bacteria, enteric bacteria, chemical properties, lake water

INTRODUCTION

Water has always been a vital item for man's existence. Man uses water for many purposes like drinking, irrigation, fisheries, industrial process, transportations and waste disposal (Kumar and Kakrani 2000). The most common aquatic systems of Bangladesh are ponds, rivers, beels, haors, baors and lakes which are fresh water resources upon which people depend directly or indirectly. This resource has become worsen due to over population, urbanization, industrialization and pollution. Pollution is introduced by man into the environment of substances of energy liable to cause hazards to human health, harm to living individuals and ecological systems, damage to structure or amenity or interference with legitimate uses of the environment (Holdgate 1979). A wide variety of inorganic and organic compounds and microorganisms causes pollution, often play a major role in determining the extent of this pollution (Higgnis and Burns 1975). Organic pollution occurs when large quantities of organic compounds are released into water. Organic pollutant

*Author for correspondence: Email: <makarim10@yahoo.com>. [1]Department of Soil, Water and Environment, University of Dhaka, Dhaka 1000, Bangladesh.

consists of protein, carbohydrate, fats and nucleic acids, which originate from domestic sewage, urban run-off, industrial effluents etc. and act as substrates for microorganisms (Mason 2002). In 1997, Godfree and coworkers mentioned that fecal streptococci is an indicator of fecal contamination in water. Indicator microorganisms, such as total coliform, fecal coliform and fecal streptococci have been used as a model for the potential presence of pathogenic microorganisms (Patra *et al.* 2009). Factors responsible for the pollution of lake water are direct discharge of untreated sewage and dumping of solid wastes into the lake. Besides, surface run-off during the rainy season that carries pollutants from the catchment areas of the lake, fragmentation of lake ecosystem by culverts that hinders free movement of water, lack of effective conservation activities of the lake ecosystem etc., contribute to the lake pollution. Considering above situation and facts, present work was undertaken to determine aerobic heterotrophic, enteric and related bacteria to the context of biological pollution level along with physicochemical properties to reveal an overall status of pollution of the Gulshan lake water.

MATERIAL AND METHODS

Study site and sampling: Water samples were collected in clean carbon polypropylene containers from four different sites (viz. GL-1, GL-2, GL-3 and GL-4) of the lake (Fig. 1). The lakeside, where floating people used to take their bath, swim and wash clothes in the lake water, were selected as the sampling sites.

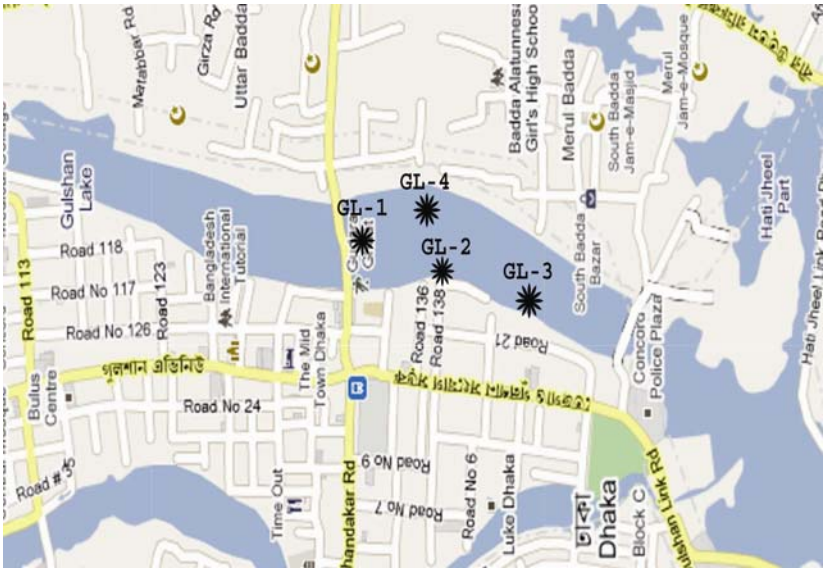

Fig. 1. Sampling sites of the Gulshan lake.

Isolation of bacteria: Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria. MacConkey agar (Difco), SS agar (Diagnostic Pasteur) and Cetrimide agar (Difco) (for enteric bacteria) media were used for the determination and isolation of enteric bacteria. The pH of the isolation media was adjusted to 7.2 before sterilization, since the pH values of most of the samples were within the range of 6.4 to 7.6. Three different techniques, *viz*. serial dilution plate (Clesceri *et al*. 1998), spread plate (Sharp and Lyles 1969) and membrane filtration techniques (Atlas *et al*. 1995) were used for the enumeration and isolation of bacteria. Inoculated bacterial plates were inverted and incubated at 37°C for 24 hours.

Bacterial colony counting was made with the help of a digital colony counter (OSK 10086, DC-3, Kayagaki, Japan). Discrete bacterial colonies were isolated immediately after counting. In case of MacConkey agar medium, pink or brick red colonies were considered as lactose fermenter and colorless colonies as non-lactose fermenter.

Physico-chemical properties of water: Temperature of water samples was measured at the time of water sampling with the help of a mercury thermometer. The pH of collected water samples was measured by using a digital pH meter (Jenway 3310, U.K.) immediately after collecting the samples. For chemical analysis, water samples were passed through filter paper (Whatman No. 42, England) to eliminate suspended solid particles. Nitrite-nitrogen present in the water samples was determined by the modified Griess-Ilosvay method (Barnes and Folkard 1951, Bremner 1965). The nitrate-nitrogen of the water samples was determined calorimetrically (Joergensen and Brookes 1990). Soluble reactive phosphorous was determined by ascorbic acid blue color method (Murphy and Riley 1962). To determine ammonium nitrogen, the water samples were distilled under alkaline condition in a Micro-Kjeldahl apparatus (Page *et al*. 1982).

The Dissolved Oxygen (DO) of the water sample was determined by a DO meter (Jenway 970 DO_2, Bibby Scientific Limited, Staffordshire, UK), while Chemical Oxygen Demand (COD) was analyzed based on chemical oxidation of materials in the presence of catalyst $Cr_2O_7^{2-}$ in 50% H_2SO_4 (De 2001). The conductivity of the water samples was measured with the help of a conductivity meter (Hanna, MODEL-HI 9033). Total Dissolved Solids (TDS) were measured with the help of a TDS meter (Hanna, MODEL-HI 9034), while the alkalinity of the water samples was determined by the titrimetric method.

Identification of bacterial isolates: Important physiological and biochemical characteristics were studied for the identification of the selected isolates. Different biochemical tests, *viz*. catalase, oxidase, carbohydrate fermentation,

deep glucose agar, tyrosine degradation, egg-yolk lecithinase, arginine hydrolysis, casein hydrolysis, starch hydrolysis, gelatin hydrolysis, protease, urease, Kligler's iron agar (KIA), Levan, methyl red, nitrate reduction, indole production, phenylalanine deamination, citrate utilization, utilization of propionate, Voges-Proskauer (V.P) test etc. were carried out. The Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986) was followed for the identification of Gram-positive aerobic heterotrophic bacterial isolates, while Manual for the laboratory investigations of acute enteric infections (WHO 1987) and Bergey's manual of systematic bacteriology (Krieg and Holt 1984) were used for identifying enteric and related bacteria.

RESULTS AND DISCUSSION

The physical properties of the water samples are given in Table 1. In Summer, the water temperature ranged between 22 and 24°C, while in Rainy season that was 25°C. In winter, the temperature ranged between 11 and 12°C and in late Autumn, it was found between 28.1 and 29.9°C. The results indicate a favourable temperature for bacterial growth except in Winter. The pH of the water samples ranged between 7.26 and 7.5 in Summer, 7.34 and 7.61 in Rainy season, 8.27 and 8.37 in Winter and 6.41 and 7.55 in late Autumn. The maximum pH (8.37) was detected in the sample GL-2 in Winter, while the minimum pH (6.41) was seen in the sample GL-3 of late Autumn (Table 1). Amount of NH_4^+-N varied between the samples with the seasons. The maximum NH_4^+-N (0.8 mg/l) was detected in GL-2 during late Autumn, while minimum

Table 1. Average temperature and pH of the water samples of the Gulshan lake.

Sampling station	Summer		Rainy season		Winter		Late autumn	
	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH	Temperature (°C)	pH
GL-1	24	7.29	25	7.41	12	7.32	29.9	7.32
GL-2	22	7.32	25	7.56	11	7.37	29.6	7.33
GL-3	23	7.26	25	7.61	11	7.27	28.1	6.41
GL-4	24	7.50	25	7.34	11	7.29	29.6	7.55

(0.003 mg/l) was in the samples GL-1, 3 and 4 during Winter (Table 2). Naturally occurring NH_4^+-N levels in ground and surface waters are usually bellow 0.2 mg/l, but toxicological effects are observed above 200 mg/kg of body weight (De 2001). Higher amount (60 mg/l) of NO_3^--N was detected in the sample GL-1 during Winter, while minimum was recorded in all the samples during late Autumn (Table 2). In case of NO_2^--N, higher value (15 mg/l) was recorded in the sample GL-1 during late Autumn and lower value (0.15 mg/l) was in the

Table 2. Chemical properties of the water samples of the Gulshan lake.

Sampling station	DO (mg/l)	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	Conductivity (µs/cm)	Alkalinity (meq/l)	NO_2^--N (mg/l)	NO_3^--N (mg/l)	NH_4^+-N (mg/l)	PO_4- (mg/l)
Summer										
GL-1	1.2	0.9	ND	288	549.5	5.95	ND	ND	ND	ND
GL-2	2.34	2.33	ND	294	515.5	5.95	ND	ND	ND	ND
GL-3	1.88	1.88	ND	295	543	5.85	ND	ND	ND	ND
GL-4	3.14	2.98	ND	293	540	6.00	ND	ND	ND	ND
Rainy season										
GL-1	1.45	1.20	ND	218	1303	4.15	ND	ND	ND	ND
GL-2	1.26	1.11	ND	216	1328	4.25	ND	ND	ND	ND
GL-3	1.35	1.19	ND	220	1319	4.30	ND	ND	ND	ND
GL-4	1.16	0.96	ND	220	1308	4.10	ND	ND	ND	ND
Winter										
GL-1	2.23	1.616	63.42	261.6	500.50	4.85	0.16	60	2.7×10^{-3}	2.0
GL-2	2.80	2.34	39.03	267	486	5.00	0.15	26	5.2×10^{-2}	1.98
GL-3	0.55	0.16	112.20	264	506.30	4.70	0.16	25.6	2.7×10^{-3}	2.30
GL-4	0.63	0.319	48.78	269.6	492	4.90	0.16	40	2.7×10^{-3}	2.25
Late Autumn										
GL-1	1.88	ND	58.54	211.3	414	3.80	15.0	Trace	1.8×10^{-2}	1.15
GL-2	2.19	ND	48.78	213.3	415.30	4.10	6.5	Trace	8.1×10^{-1}	1.20
GL-3	3.35	ND	ND	226.6	435	3.10	6.4	Trace	ND	1.25
GL-4	1.28	ND	48.78	215.3	402.60	3.90	10.0	Trace	2.7×10^{-2}	2.00

ND = Not Done

sample GL-2 of Winter. Higher concentration of phosphorus (2.3 mg/l) was observed in the sample GL-3 during Winter, while lower value (1.25 mg/l) was in the same site during late Winter (Table 2). According to the United States Public Health (USPH), drinking water standard is pH 6.0 to 8.5, NH_4 0.5 mg/l, NO_2-N <10 mg/l, NO_3 <10 mg/l to 45 mg/l (De 2001).

In the present study, the values of NH_4^+ were much lower than NO_3^- and NO_2^- that was a trend to nitrification process during both Winter and late Autumn. The value of the total amount of nitrogen (NH_4, NO_3^- and NO_2^-) and phosphorus were found to be satisfactory from the pollution point of view with the guidelines recommended by USPH (Table 2).

According to USPH standard, 4 mg/l and 5 mg/l values of BOD and COD, respectively indicate quality for domestic and drinking water (De 2001). The normal range of BOD for good water quality is 5-6 mg/l and COD is 6-10 mg/l (Haq *et al.* 2005). Higher COD values indicate that the water of the Gulshan lake was considerably polluted with chemical pollutants (Table 2). Natural level of nitrate-nitrogen in surface water is usually below 60 mg/l. Extensive epidemiological data do not support the current guideline values for NO_3-N of 10 mg/l (WHO 1996).

The aerobic heterotrophic and enteric bacterial count of the water samples was shown in Table 3. Maximum aerobic heterotrophic bacterial load (6×10^6 cfu/100 ml) was in GL-1 during Summer, while minimum (8×10^5 cfu/100 ml) was in GL-4 during Winter. Bacterial count on MacConkey agar ranged between 0 cfu/100 ml and 2×10^7 cfu/100 ml, where maximum count was observed in the sample GL-1 during Rainy season posing faecal contamination from the bank of the lake through rain water. The highest count of bacteria (3×10^5 cfu/100 ml) on SS agar was found in GL-4 during Summer, while the lowest count (1×10^3) was found in GL-3 during late Autumn. However, no colony was formed in the sampling sites GL-1, 2 and 3 during Rainy season and all sites during Winter. Bacterial count on Cetrimide agar ranged between 0 and 1.4×10^3 cfu/100 ml (Table 3).

During this investigation of the 40 isolates, 14 were randomly selected and purified for provisional identification. Selected isolates were identified on the basis of their morphological characters, gram reaction and necessary biochemical tests (Table 4). Among these seven were Gram-positive and remaining seven were Gram-negative. Among the Gram-positive, four isolates were rod shaped, spore former and members of the genus *Bacillus,* and the remaining three were the members of *Planococcus citreus* and *Lactobacillus plantarum*. Under the genus *Bacillus*, there were four distinct species, *viz. B. polymyxa*, *B. subtilis*, *B. anthracis* and *B. alvei*. Enteric bacteria were Gram-

negative, short rod and non-spore former. Seven Gram-negative bacterial isolates were under the genera *Pseudomonas* (2 spp.), *Neisseria*, *Morganella*, *Escherichia*, *Aeromonas* and *Enterobacter* (Table 4). The load of aerobic heterotrophic bacteria and abundance of coliform and faecal coliform group in the water clearly showed a significant level of the microbial pollution of the lake water. The coliform group of bacteria including aerobic and facultative anaerobic bacilli that produce acid and gas from the fermentation of lactose includes *Escherichia coli* and *Enterobacter aerogenes* (Maier *et al*. 2000). Both *E. coli* and *Enterobacter* sp. were present in the lake water samples during the present study. *Psudomonas aeruginosa* has been employed as sewage indicator, while *Aeromonas hydrophila* as an indicator of eutrophication (Bahlaoui *et al*. 1997). Presence of *E. coli* and *Pseudomonas* sp. in the present study clearly indicates that the lake water is contaminated with fecal and sewage pollution. The lake is being used as bathing, washing and swimming by the floating people, which is likely to be infected with these type of pathogens and this may cause waterborne diseases like diarrhoea, typhoid, dysentery etc. Furthermore, cultivated fish could also be contaminated with those pathogens that might be transmitted to the people and can cause waterborne diseases.

Table 3. Bacterial count (cfu/100 ml) of the water samples of the Gulshan lake.

Sampling sites	Aerobic heterotrophic bacteria	Enteric and related bacteria on			Total bacterial load
		MacConkey agar	SS agar	Cetrimide agar	
Summer					
GL-1	6×10^6	1×10^6	15×10^4	–	7.2×10^6
GL-2	1×10^6	2×10^5	5×10^4	–	1.25×10^6
GL-3	2×10^6	1×10^7	7×10^4	–	12.1×10^6
GL-4	4×10^6	1×10^6	3×10^5	–	5.3×10^6
Rainy season					
GL-1	9×10^6	2×10^6	1×10^5	1×10^2	11.1×10^6
GL-2	5×10^6	1×10^6	–	–	6×10^6
GL-3	1×10^6	–	–	3×10^2	1×10^6
GL-4	4×10^6	–	–	–	4×10^6
Winter					
GL-1	2×10^6	4×10^4	–	7×10^2	2.04×10^6
GL-2	3×10^6	4×10^5	–	2×10^3	3.4×10^6
GL-3	3×10^6	–	–	3×10^2	3×10^6
GL-4	8×10^5	1×10^4	–	–	0.81×10^6
Late Autumn					
GL-1	ND	NC	2×10^4	–	0.02×10^6
GL-2	14×10^5	NC	1×10^4	–	1.41×10^6
GL-3	ND	NC	1×10^3	–	0.001×10^6
GL-4	15×10^5	NC	4×10^4	1×10^2	1.54×10^6

ND = Not done, " – " = No colony, NC = Not counted.

Table 4. Biochemical characteristics and provisional identification of the selected bacterial isolates from the water of the Gulshan lake.

Isolate No.	Citrate utiliza-tion	Oxidase	Catalase	VP	MR	Tyrosine degra-dation	Casein hydro-lysis	Starch hydro-lysis	Gelatin hydro-lysis	Nitrate reduction	Provisionally Identified name
LW-4	–	+	+	+	+	–	+	–	+	+	*Morganella morganii*
LW-7	–	–	+	+	–	–	+	–	+	–	*Neisseria flavescens*
LW-11	+	+	+	–	+	+	+	+	+	+	*Pseudomonas saccharophila*
LW-13	–	–	+	+	+	–	+	+	+	+	*Bacillus polymyxa*
LW-14	–	+	+	+	+	–	+	–	+	+	*B. subtilis*
LW-15	+	–	+	+	+	–	+	+	+	+	*Lactobacillus plantarum*
LW-19	+	+	+	+	+	+	+	D	+	+	*L. plantarum*
LW-21	–	–	+	+	+	–	+	+	+	+	*Planococcus citreus*
LW-26	–	+	+	+	+	+	+	+	+	+	*B. anthracis*
LW-29	–	+	+	+	+	+	+	+	+	+	*B. alvei*
LW-33	–	–	–	–	–	–	–	–	–	–	*Escherichia coli*
LW-37	+	+	+	–	–	+	+	–	+	+	*Psudomonas aeruginosa*
LW-42	–	–	+	–	+	–	–	–	–	–	*Aeromonas hydrophila*
LW-49	–	–	–	–	+	–	–	–	–	–	*Enterobacter* sp.

"+" indicate positive, "–" indicate negative and "D" Doubtful

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