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BACTERIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF THE GULSHAN LAKE, DHAKA

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Abstract

Seasonal variation on bacterial load irrespective of heterotrophic and enteric bacteria was noticed in Gulshan lack of Dhaka Metropolitan city. The lowest number (1.56×10^6 cfu/100 ml) in the summer and the highest (19.03×10^6 cfu/100 ml) were recorded during winter. Among the isolated bacteria 32 were studied in details and provisionally identified as *Bacillus cereus* (1), *B. sphaericus* (1), *B. subtilis* (1), *B. brevis* (2), *B. pasteuri* (1), *B. stearothermophilus* (1), *B. azotoformans* (1), *B. pantothenticus* (1), *B. licheniformis* (1), *B. circulans* (1), *B. insolitus* (1), *Planococcus citrius* (1), *Micrococcus sedentarius* (1), *Pseudomonas. syringae* (4), *P. aeruginosa* (1), *P. cichorii* (1), *Escherichia* (2), *Klebsiella* (1), *Aeromonas* (1), *Proteus* (1), *Enterobacter* (1), *Salmonella* (2), *Yersinia* (1), *Shigella* (1), *Hafnia* (1), and *Alcaligens* (1). The temperature and pH of the water samples varied between 24.3 and 29.2º C and 6.54 and 7.12, respectively. Chemical contents (mg/l) like NH_4^+-N, NO_3^--N and phosphorus ranged 3.06 (Winter) to 14.86 (Summer), 0.32 (in Rainy season) to 13.02 (Summer) and 0.81 (Rainy season) to 1.86 (Summer), respectively. The enteric bacterial load (0.03×10^5 cfu/100 ml in Rainy season to 21.2×10^5 cfu/100 ml in Winter) and the presence of *Escherichia, Aeromonas, Enterobacter, Pseudonomas* in the samples indicated significant level of microbial pollution of the lake.

Introduction

Aquatic microorganisms and their activities are of great importance in many ways. They may affect the health of humans and other animals. These organisms occupy a key position in the food chain by providing rich nourishment for the next higher level of aquatic life and may affect health of humans and other animals (Pelczar 1988). Water quality is a broad concept. Its maintenance means that natural waters should not be overloaded with organic or inorganic nutrients or with toxic, noxious, or esthetically unacceptable substances. They should not become vehicles of disease transmission from fecal contamination nor should their oxygenation, temperature, salinity, turbidity, or pH be altered significantly (Atlas and Bartha 1998).

The Gulshan lake is one of the important artificial lakes of Dhaka Metropolitan city. It is situated in posh area of Dhaka but at the same time slum people are frequently using this lake water for bathing and cooking and also polluting the water body. Pollution also may be caused by parking lots, gardens, driveways, sidewalks, lawns, and roads, agricultural works and fish farming in the lake results in deterioration of its water quality. Pollution can be caused by wide variety of inorganic and organic compounds and microorganisms 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 courses. Organic pollutant consists of protein, carbohydrate, fats and nucleic acids which originate from domestic sewage, urban run off, industrial effluents etc. They act as substrates for microorganisms. (Mason 2002). Outbreaks of water-borne diseases continue to occur in both developed and developing countries leading to loss of life and economic burdens for individuals and communities (WHO 1993). The coliform groups of bacteria in general and *E. coli* in particular have found universal application as indicators of

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fecal contamination. Godfree *et al.* (1997) mentioned that fecal streptococci as indicators of fecal contamination in water. Fecal bacteria can be liberated from various sources, including agricultural sources, wild and domesticated animals, urban development and effluent treatment facilities (Kelsey *et al*. 2004). Indicator microorganisms such as total coliform, fecal coliform and fecal streptococci have been used as models for the potential presence of pathogenic microorganisms in water samples (Patra *et al*. 2009).

In Bangladesh, about 80% of all diseases are associated with water-borne microbes. About 300,000 children under five, die of diarrhoeal diseases every year, out of which one-third of the death occurs in the city slums and squatter settlements (GOB-UNICEF 1991). The physicochemical features and bacterial flora of Dhanmondi lake were investigated earlier (Khondker and Parveen 1992, Saha *et al.* 2002). The present study was undertaken to enumerate aerobic heterotrophic, enteric and related bacteria and physico-chemical properties to find out an overall pollution of the Gulshan lake.

Materials and Methods

Samples were collected from the five selected sites of Gulshan lake at three different seasons. Water samples were collected in plastic bottles sterilized with alcohol.

Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria present in water samples. The pH of the medium was adjusted to 7.2 since the pH values of most of the samples were within the range of 6.7-7.5. For the determination and isolation of enteric and related bacteria, MacConkey agar medium (Difco) and SS agar medium (Diagnostic Pasteur) were used. Three different techniques were used *viz.* dilution plate technique (APHA 1998), spread plate technique (Sharp and Lyles 1969) and membrane filtration technique (Atlas *et al.* 1995) for the enumeration and isolation of bacteria (APHA 1998). The inoculated plates were inverted and incubated at 37° C for 24 hrs in an incubator. After 24 hrs of incubation the plates having discrete colonies were counted with a colony counter (Digital colony counter, DC-8 OSK 100086, Kayagaki, Japan). In case of MacConkey agar medium, pink or brick-red colonies were considered as lactose fermenter and colorless colonies as non-lactose fermenter. Selected bacterial colonies were isolated immediately after counting.

For provisional identification of the isolates, important biochemical tests *viz.* carbohydrate fermentation, arginine hydrolysis, catalase, deep glucose agar, tyrosine degradation, egg-yolk lecithinase, casein hydrolysis, protease, gelatin hydrolysis, starch hydrolysis, KIA, methyl red, nitrate reduction, citrate utilization, oxidase, urease etc. were carried out. Bergey's Manual for Systematic Bacteriology (Sneath *et al*. 1986) was followed for the identification of Gram-positive bacterial isolates. The enteric and related bacteria were identified by using standard method following Manual for Laboratory Investigations of Acute Enteric Infections (WHO 1987) and Bergey's Manual for Systematic Bacteriology (Krieg and Holt 1984).

The pH of water samples was measured in the laboratory by using a pH meter (Jenway 3310 pH meter, U.K.) immediately after collection. Samples were then preserved in a refrigerator at 4º C before and after the microbiological and physicochemical analyses. For chemical analysis, samples were passed through the filter paper (Whatman No. 40, England) to remove coarse particles. Chemical analysis was carried out quickly after sampling. To determine ammonium nitrogen, water samples were distilled under alkaline condition in a micro Kjeldahl distillation apparatus (Jackson (1973). Nitrate nitrogen of the water samples was determined colorimetrically (Cataldo *et al*. 1975). Phosphorus content was determined by ascorbic acid blue color method (Murphy and Riley 1962).

Results and Discussion

The bacterial count of the water samples of the Gulshan lake showed substantial number of aerobic heterotrophic and enteric bacteria (Table 1). Maximum aerobic heterotrophic bacterial load (19.0×10^6 cfu/100 ml) during Winter and minimum (1.5×10^6 cfu/100 ml) were found in the sample Gl-5 during Summer. Bacterial count on MacConkey agar ranged between 0.03×10^5 cfu/100 ml and 21.2×10^5 cfu/100 ml. The lowest number of bacteria (0.01×10^5 cfu/100 ml) on SS agar medium was recorded in the sample Gl-3 during Winter season while the highest number (0.19×10^5 cfu/100 ml) was in the sample of Gl-2 in Rainy season. The total (aerobic heterotrophic and enteric) bacterial count ranged between 1.56×10^6 and 19.03×10^6 cfu/100 ml.

Table 1. Bacterial count (cfu/ 100 ml) of water samples of Gulshan lake.

Season	Sampling sites	Aerobic heterotrophic bacteria on nutrient agar	Enteric bacteria on		Average enteric bacteria	Total bacterial load
			MacConkey agar	SS agar		
Summer	Gl-1	7.0×10^6	2.0×10^5	0.18×10^5	1.1×10^5	7.11×10^6
	Gl-2	13.0×10^6	0.2×10^5	0.13×10^5	0.17×10^5	13.02×10^6
	Gl-3	10.3×10^6	6.9×10^5	0.16×10^5	3.53×10^5	10.65×10^6
	Gl-4	2.4×10^6	0.9×10^5	0.08×10^5	0.49×10^5	2.45×10^6
	Gl-5	1.5×10^6	1.0×10^5	0.11×10^5	0.56×10^5	1.56×10^6
Rainy	Gl-1	10.7×10^6	0.1×10^5	0.08×10^5	0.09×10^5	10.71×10^6
	Gl-2	15.0×10^6	3.0×10^5	0.19×10^5	1.60×10^5	15.16×10^6
	Gl-3	13.0×10^6	0.2×10^5	0.12×10^5	0.16×10^5	13.02×10^6
	Gl-4	13.9×10^6	0.03×10^5	0.15×10^5	0.09×10^5	13.91×10^6
	Gl-5	14.8×10^6	0.1×10^5	0.16×10^5	0.13×10^5	14.81×10^6
Winter	Gl-1	8.2×10^6	12.4×10^5	0.07×10^5	6.24×10^5	8.12×10^6
	Gl-2	11.8×10^6	21.2×10^5	0.04×10^5	10.62×10^5	12.86×10^6
	Gl-3	18.7×10^6	2.0×10^5	0.01×10^5	1.01×10^5	18.8×10^6
	Gl-4	19.0×10^6	0.4×10^5	0.13×10^5	0.27×10^5	19.03×10^6
	Gl-5	11.2×10^6	0.9×10^5	0.08×10^5	0.49×10^5	11.25×10^6

During this study, a total of 54 colonies were primarily selected and finally 32 isolates were provisionally identified on the basis of their morphological characters, gram reaction and biochemical tests (Table 2). Out of these 32 isolates, 20 were aerobic heterotrophic bacteria and remaining 12 were enteric and related bacteria. Out of 20 aerobic heterotrophic isolates, 14 were Gram-positive bacteria and belonged to 3 genera *viz. Bacillus*, *Planococcus* (*Planococcus citrius*), and *Micrococcus* (*Micrococcus sedentarius*). Under the genus *Bacillus* there were 12 distinct species, *viz. B. cereus* (1), *B. sphaericus* (1), *B. subtilis* (1), *B. brevis* (2), *B. pasteuri* (1), *B. stearothermophilus* (1), *B. azotoformans* (1), *B. pantothenticus* (1), *B. licheniformis* (1), *B. circulans* (1), and *B. insolitus* (1). The remaining 6 Gram-negative isolates belong to the genera *Pseudomonas.* Under the genus *Pseudomonas* there were 3 distinct species *viz. P. syringae* (4), *P. aeruginosa* (1) and *P. cichorii* (1). All the 12 enteric related bacteria belonged to 10 genera *viz.*, *Escherichia* (2), *Klebsiella* (1), *Aeromonas* (1), *Proteus* (1), *Enterobacter* (1), *Salmonella* (2), *Yersinia* (1), *Shigella* (1), *Hafnia* (1), and *Alcaligens* (1) (Table 2).

Table 2. Provisional identification of the selected bacterial isolates of Gulshan lake.

Isolate Nos.	Casein hydrolysis	Gelatin hydrolysis	Starch hydrolysis	Urease	Tyrosine degradation	VP	MR	Nitrate reduction	Oxidase	Identified bacteria
NA-18	+	+	+	-	-	+	-	+	-	*Bacillus cereus*
NA-17	+	+	-	-	-	-	-	-	+	*B. sphaericus*
NA-2	+	+	+	+	-	+	-	+	+	*B. subtilis,*
NA-5, NA-13	-	+	-	+	+	-	-	-	-	*B. brevis*
NA-11	+	-	-	-	-	-	-	+	+	*B. pasteurii*
NA-12	+	+	+	-	-	-	-	+	+	*B. stearothermophilus*
NA-6	+	-	-	-	-	-	-	-	+	*B. azotoformans*
NA-14	-	+	+	-	-	-	-	+	-	*B. pantothenticus*
NA-15	+	+	+	-	-	+	-	+	-	*B. licheniformis*
NA-16	+	+	+	-	-	-	-	+	-	*B. circulans*
NA-20	-	-	-	-	-	-	-	-	-	*B. insolitus*
NA-1	-	+	+	-	+	+	-	+	+	*Planococcus citrius*
NA-3	+	+	-	-	-	+	-	+	+	*Micrococcus sedentarius*
NA-4, NA-7, NA-9, NA-10	-	+	+	+	-	-	+	-	-	*Pseudomonas syringae*
NA-8	+	+	-	+	+	-	-	+	+	*P. aeruginosa*
NA-19	-	-	+	+	+	-	-	-	-	*P. cichorii*
MA-1, MA-3	-	-	-	-	-	-	-	-	-	*Escherichia coli*
MA-2	-	-	-	+	-	-	-	-	-	*Klebsiella* sp.
MA-4	-	-	-	+	-	-	+	-	-	*Aeromonas hydrophila*
MA-5	-	-	-	-	-	+	-	-	-	*Proteus morganii*
MA-6	-	-	-	-	-	-	+	-	-	*Enterobacter* sp.
SS-1, SS-2	+	+	-	-	+	-	-	+	+	*Salmonella* sp.
SS-3	-	-	-	-	+	-	-	+	+	*Yersinia* sp.
SS-4	-	-	-	-	-	-	-	-	+	*Shigella* sp.
SS-5	-	-	-	-	-	+	-	+	-	*Hafnia* sp.
SS-6	-	-	-	-	-	-	-	-	+	*Alcaligens* sp.

The coliform group of bacteria includes all the aerobic and facultative anaerobic, Gram-negative non-sporulating bacilli that produce acid and gas from the fermentation of lactose. The classical species of this group are *Escherichea coli* and *Enterobacter aerogenes* (Pleczar *et al.* 1988). In this study both the species *Escherichea coli* and *Enterobacter* sp. were recorded from the lake water samples. Saha *et al*. (2002) reported more or less similar type of bacteria in the Dhanmondi lake. The concept of indicator organisms in water microbiology is well established. The coliform group of bacteria in general and *E. coli* in particular, has found universal application as indicators of faecal contamination. In India, Patra *et al.* (2009) showed positive relationships between fecal indicators and pathogenic microorganisms. On the other hand *Pseudomonas aeruginosa* has been employed as sewage indicators and *Aeromonas hydrophila* as an indicator of eutrophication (Godfree *et al*. 1997). Presence of *E. coli*, *Pseudomonas* in this study clearly indicated the above mentioned indicators of faecal and sewage pollution. The Gulshan lake is being used as bathing and swimming by the floating people of Dhaka city and which is likely to be infected with these type of waterborne pathogens and this may cause too many gastrointestinal diseases like diarrhea, dysentery, typhoid etc.

The physico-chemical properties of the samples were shown in Table 3. In Summer the water temperature ranged between 27.8 and 29.2º C, while in Winter it was found between 24.3 and 26.2 ºC. In Rainy season the temperature ranged between 27.4 and 28.5º C. The result indicated a favorable temperature for bacterial growth. The pH of the samples ranged between 6.78 and 7.12

Table 3. Physico-chemical properties of the water samples of Gulshan lake.

Season	Sampling sites	Temperature (ºC)	pH	Ammonium–N (mg/l)	Nitrate–N (mg/l)	Phosphorus (mg/l)
Summer	Gl-1	29.0	6.99	12.04	13.02	1.54
	Gl-2	28.4	7.05	14.86	0.65	1.23
	Gl-3	29.2	6.78	7.03	1.95	0.98
	Gl-4	27.8	7.05	5.76	3.26	1.45
	Gl-5	28.2	7.12	13.73	0.39	1.86
Rainy	Gl-1	27.4	6.54	10.81	5.12	0.89
	Gl-2	27.8	7.02	8.62	0.32	0.94
	Gl-3	28.1	6.62	13.14	2.20	0.81
	Gl-4	27.8	6.88	10.03	5.45	1.12
	Gl-5	28.5	7.05	9.18	1.26	1.16
Winter	Gl-1	26.2	7.03	4.61	10.44	1.48
	Gl-2	24.5	7.02	12.31	1.041	1.37
	Gl-3	26.0	6.79	4.11	4.69	1.28
	Gl-4	25.5	6.90	3.06	1.30	1.16
	Gl-5	24.3	7.11	3.87	2.60	1.22

in Summer, 6.54 and 7.05 in Rainy season and 6.79 and 7.11 in Winter. The maximum pH (7.12) was found in the sample Gl-5 in Summer, while the minimum pH (6.54) was recorded in the sample Gl-1 during Rainy season. Amount of NH_4^+-N varied between samples with the seasons. The maximum NH^+-N (14.86 mg/l) was recorded during Summer in the sample Gl-2 and the minimum (3.06 mg/l) was in the sample Gl-4 during Winter. Ammonia in water is an indicator of possible sewage and animal waste pollution. Natural levels in ground and surface wastes are usually bellow 0.2 mg/l, but toxicological effects are observed only at exposures above 200 mg/kg of body weight (De 1989). Higher amount (13.02 mg/l) of NO_3^--N was detected in sample Gl-1 in summer while minimum (0.32 mg/l) was observed in the sample Gl-2 during rainy season. Naturally occurring nitrate levels in surface water and groundwater generally contain a few milligrams per litre. On the other hand extensive epidemiological data support the current guideline values for NO_3^--N of 10 mg/l (WHO 1993). In this study, the levels of nitrogen (NH_4^+-N and NO_3^--N) were found lower than the normal levels in most of the cases. Higher concentration of phosphorus (1.86 mg/l) was recorded in the sample Gl-5 in Summer while minimum was recorded in the sample Gl-3 during rainy season. According to the United State Public Health (USPH), drinking water standards are pH 6.0 to 8.5, NH_4 0.5 mg/l, NO_3<10 mg/l to 50 mg/l (De 1999).

During the present study, value of major chemical species like NO_3^--N, NH_4^+-N and phosphorus were found to be satisfactory from pollution point of view except for one sample compared with the guideline values recommended by USPH. Many microorganisms are able to use ammonia as a nitrogen source for cellular nutrition. Nitrifying organisms derive energy from the oxidation of ammonia to nitrate but high levels of ammonia and pH in waste waters may inhibit nitrification and thus cause persistence of accumulation of ammonia and/or nitrite (WHO 1987). The load of aerobic heterotrophic bacteria and the presence and abundance of *Escherichia*, *Aeromonas*, *Enterobacter*, *Pseudonomas*, etc. in the water samples clearly showed significant level of microbial pollution of the lake.

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