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Authors: Rahman, Md Habibur, Ferdouse, Jannatul, Ullah, A K M Atique, and Hossain, Md Towhid

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Md Habibur Rahman[1], Jannatul Ferdouse[1], A K M Atique Ullah[2] and Md Towhid Hossain[1]

[1]University of Chittagong, Bangladesh, and [2]Bangladesh Atomic Energy Commission, Kazi Nazrul Islam Avenue, Shahbag, Dhaka, Bangladesh

ABSTRACT: The present study was undertaken to determine a holistic picture of pollution comprising the physicochemical and microbiological properties of the Halda River water of Bangladesh. A total 45 samples were collected from five sampling sites. Different water quality parameters including temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), turbidity, concentration of heavy metals, total bacterial count (TBC), total coliform (TC), and fecal coliforms (FC) were assessed. Isolation and identification of 10 bacterial isolates was done using their cultural, morphological, physiological, biochemical characteristics, and 16S rRNA gene sequencing analysis. Antibiotic sensitivity of selected isolates was also investigated. DO (6–14.67 mgL^{-1}), BOD (2–8 mgL^{-1}), COD (40.43–107.63 mgL^{-1}), TSS (818.33–3,180 mgL^{-1}), TS (1,039.33–3,897.33 mgL^{-1}), and turbidity (40.09–355 NTU), TBC (7.2×10^3–3.83×10^5 CFU/mL), FC (0.7×10^2–3.9×10^3/100 mL) were measured in both non breeding and breeding time that implies the pollution of the Halda river water. Heavy metal concentration was found within standard limit. Among the isolates, *Bacillus wiedmannii* strain HSA2 (accession no. MT102620) showed multidrug-resistant, which is a matter of great concern. According to the study findings, the water quality of the Halda river is being deteriorated, posing a threat to public health as well as the aquatic environment.

KEYWORDS: Halda river, microbiological parameters, physiochemical parameters, water quality, bacterial strains

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CORRESPONDING AUTHORS: Jannatul Ferdouse, Department of Microbiology, Faculaty of Biological Sciences, University of Chittagong, Hathazari Road, Chittagong, 4331, Bangladesh. Email: ferdouse@cu.ac.bd.

Md Towhid Hossain, Department of Microbiology, Faculty of Biological Sciences, University of Chittagong, Hathazari Road, 4331, Bangladesh. Email: towhid.mbio@cu.ac.bd.

Introduction

In Bangladesh, ground and surface water are the primary sources of water and Halda River is the most important source of surface water (Hasan et al., 2019). Among the significant primary sources of water in Bangladesh, the Halda River is very special for Indian carp fish's such as *Catla catla, Labeo rohita, Labeo calbasu, Cirrhinus mrigala*, etc. Indian carps naturally breed only in this tidal river (Patra & Azadi, 1985; Tsai et al., 1981). A number of factors like seasonal, environmental and hydro-morphological characteristics favor for spawning of carps. Studies showed that the interaction of various factors make a different hydrological condition which probably stimulate and prompt the carp breeding in the Halda River (Patra & Azadi, 1985). In addition, the soil profile of the Halda river contains sand 41.00% to 54.43%, silt 20.70% to 29.70%, clay 20.92% to 29.10%, organic matter 3.29% to 4.36%, and carbon 1.12% to 2.52% (Fontana, 2022).

Similar to other rivers, the water of Halda is used for various purposes including recreation, transportation, household uses, drinking water supply, fishing, carrying off, and assimilation of wastes from agricultural lands, domestics, municipalities, industries, streets, and roads run-off (Kabir et al., 2013). The Halda River become undesirably affected due to its direct link to the Karnaphuli River which is vastly polluted by the wastes from various sources such as municipalities and industries (Ali et al., 2016; Karim et al., 2019). In addition, anthropogenic activities (Hatje et al., 1998) factors threatening the Halda River lives (Azadi & Alam, 2013). Consequently, the natural carp fish breeding capacity of the Halda River is adversely disrupted.

Unplanned textile mills, power plant, paper mill, tannery, etc., and their accidental use of chemicals are the main causes of excessive metals in the aquatic ecosystems (Ahmed et al., 2019) and makes the Halda river water polluted. Department of Environment (DoE) and Water Resource Planning Organization (WARPO) regulate water pollution according to Bangladesh Water Act 2013 (Arifuzzaman et al., 2019). Moreover, the rising Bay of Bengal Sea water also enter the Halda through Karnaphuli river and destroying carps breeding (Illius, 2021). However, it is concerned that the metal contamination with some other vital factors in the water body might have been caused due to the anthropogenic sources along with the natural environment (Safiur Rahman et al., 2021). Water contamination due to the presence of organic and inorganic pollutants as well as the life-threatening microbes has become a national issue in Bangladesh. Therefore, the physicochemical and biological quality of the river water determines its utility for diverse application and the level of water pollution (Higgins & Burns, 1975).

There are few studies on the physiochemical parameters of Halda River water in Bangladesh. Moreover, there is also a lack of microbiological study on Halda River water. Therefore, a combined study, comprising both physiochemical and microbiological quality of this water source is needed to control

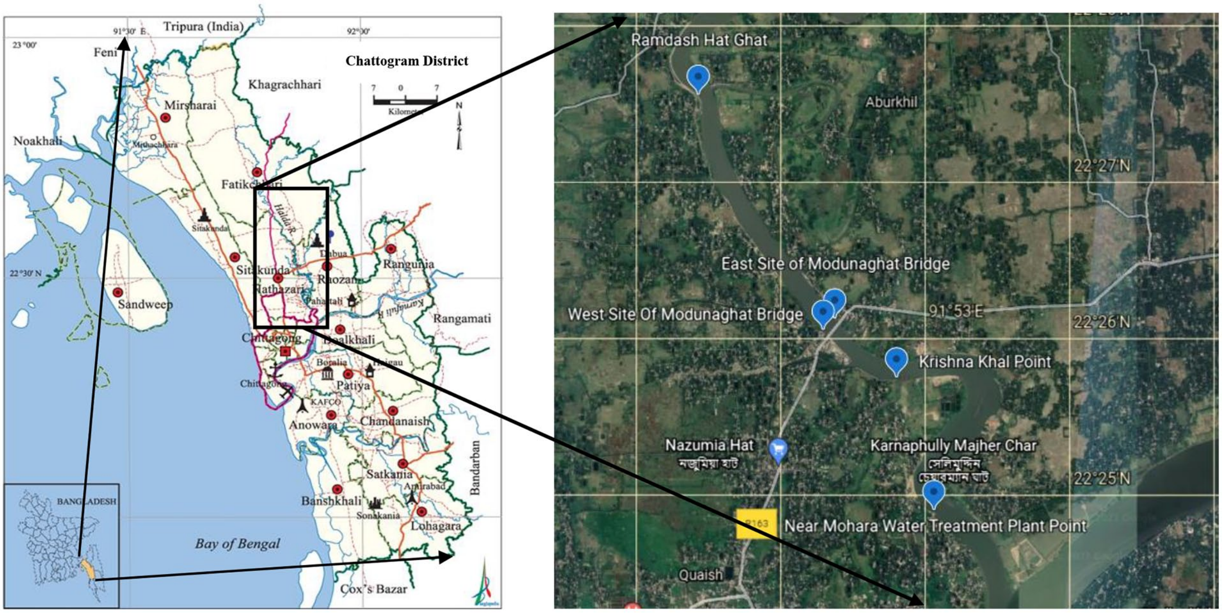

Figure 1. GPS position of Chattogram (Banglapedia, 2014) and GPS position of sampling sites.

pollution and manage sustainable water resources for aquaculture and ecosystem management.

The primary aim of this study was to assess the microbiological contamination, concentration of metals like- lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and physicochemical properties such as temperature, pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), and Total Dissolved Solids (TDS) of Halda River water in Chattogram, Bangladesh. Therefore, this research work will play a great role in addressing the public health concerns and aquatic lives in the Halda River.

Materials and Methods

Sampling sites

Sampling sites were selected based on point and non-point sources of contamination, climate, etc. Five sampling sites were selected randomly considering the location as representative of the water quality parameters wished to investigate. These are Mohara water treatment plant (22°24′53″N 91°53′03″E), point of Krishna Khal (22°25′44″N 91°52′48″E), West site of Modunaghat Bridge (22°26′02″N 91°52′17″E), East site of Modunaghat bridge (22°26′06″N 91°52′22″E) and Ramdash Hat point (22°27′32″N 91°51′26″E) (Figure 1) in Chattogram, Bangladesh.

Sample collection

Water samples were collected from the Halda River in the month of February, May (spawning time), and December 2019 to have a throughout condition of this important resource. From each sampling point three replica samples were collected manually. Among them, one sample was used for microbiological analysis, one for heavy metals analysis and another for other physicochemical analysis. Clean, dry, and sterilized conical flasks were used for sampling (Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, & WPCF, 2017). For heavy metal analysis, samples were collected from the surface water layer at the main flow using 100 mL high-density polyethylene (HDPE) bottle.

Physicochemical analysis of the water samples

In this study, several physicochemical parameters were measured to determine physicochemical properties of water of the Halda River. The instrument was calibrated in accordance with the manufacturer's guideline before taking the measurements. Temperature and pH were recorded *in-situ* using portable thermometer (Mercury 305 MM thermometer, Zeal, England) and pH meter (HANNA, Model no: HI98127). EC was measured using Combometer (Hanna portable combometer, Model no: HI9813-6), DO, and BOD was determined by Azide-Winkler Method (Winkler, 1888). BOD was calculated by measuring DO initially and after 5 days of incubation at 20°C. Moreover, COD was determined by titration after oxidative reflux of the water samples with potassium dichromate ($K_2Cr_2O_7$) in an acidic (sulfuric acid) solution. TSS, TDS, and TS were measured by gravimetric method using drying and weighing technique (Baird et al., 2017).

Determination of trace metals

Spectroscopic technique was used to determine trace metals concentration. According to the techniques, water samples were digested using Ultra-pure HNO_3 (Merck Germany, highest purity level 99.98%) and filtered through Millipore cellulose nitrate filters (pore size 0.45 mm). The pH of the filtrates was measured and kept at pH < 2. Then, the amount of Pb, Cd, Cr, Ni, and Cu was analyzed with inductively coupled plasma-mass spectrometry (ICP-MS, 820-MS, Bruker).

Microbiological quantification of water samples

Serial dilution and then pour plate technique using nutrient agar (NA) medium was followed to enumerate total viable bacteria in the water samples. Counts for every dilution was taken and multiplied by the respective dilution factor to get the result in colony forming unit per milliliter (CFU/mL) (Collins et al., 2004). Most probable number (MPN) method was used to determine total coliform and fecal coliform in the water samples. Result was computed using MPN chart as total coliform number per 100 mL sample.

Isolation and identification of isolates

Firstly, 25 bacterial colonies of distinct morphology were selected from the plate that was done with serially diluted samples from selected five sampling sites. Every selected colony was repeatedly streaked on the nutrient agar plate to purify until fresh single colony was appeared and then preserved on NA slant. The selected bacterial isolates were primarily characterized on the basis of their morphological, cultural, physiological and biochemical characteristics and 16S rRNA gene sequencing analysis. All characteristics features of the selected bacterial isolates were compared with the standard description of Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) to identify the isolates.

PCR and sequencing of 16S rRNA gene

In order to 16S rRNA gene sequences amplification, PCR was carried out with the universal primers 27F (5'-AGAGTTTGA TCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTT ACGACTT-3'). The resulting PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer protocol. The purified PCR products were sequenced by the Applied Biosystems Big Dye™ Terminators v3.1 (Thermo Fisher Scientific). The sequences were submitted to National Center for Biotechnology Information (NCBI) GenBank under the accession numbers MT102620, MT102621, MT102631, MT102632, MT102625, MT102626, MT102635, MT102628, MT102636, and MT102637.

16S rRNA gene sequences analysis

The sequence similarity analysis was carried out by comparing the 16S rRNA sequences of the selected isolates with sequence data in the GenBank database of NCBI using the blast suite optimized for "Highly similar sequences" (mega blast) (Altschul et al., 1990; Johnson et al., 2008). The Ribosomal Database Project (RDP) Classifier was used to assign a taxonomic class to each bacterial sequence. The taxonomical hierarchy of the bacteria was performed using their 16S rRNA sequences by RDP Naive Bayesian rRNA Classifier Version 11.1 setting 80% confidence threshold (Cole et al., 2014).

Construction of phylogenetic tree

The sequences of 16S rRNA genes were aligned using the multiple sequence alignment program MUSCLE using the software Geneious 9.1.3 (Kearse et al., 2012). Phylogenetic and molecular evolutionary analyses were processed through the molecular evolutionary genetics analysis software MEGA 11 (Tamura et al., 2021) using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993).

Antibiotic susceptibility test

The antibiotic susceptibility of the selected 10 identified isolates were assessed using Kirby-Bauer disk diffusion method (Hudzicki, 2009) on Mueller Hinton agar plates. Susceptibility pattern was assessed using 10 commercially available antibiotics namely Cefalexin 30 µg, Ampicillin 2 µg, Amoxicillin 30 µg, Ciprofloxacin 5 µg, Ceftazidime 30 µg, Ceftriaxone 30 µg, Amikacin 30 µg, Chloramphenicol 30 µg, Meropenem 10 µg, Imipenem 10 µg. The antibiotic susceptibility of the isolates was interpreted using the guiding principles of the Clinical and Laboratory Standards Institute (CLSI, 2016).

Results

Physicochemical parameters of water samples

To assess the water quality of Halda River, different physicochemical parameters were analyzed. The seasonal variation of different physicochemical properties of the Halda River water samples are summarized in Table 1. The temperature in the study period at different points was found in the range from 22.5°C to 27.67°C and pH value of water samples was found from 6.9 to 7.53. In addition, EC value was found within the range from 50 to 283.33 μScm^{-1}. The DO, BOD, COD value of collected water samples represented a variation of 6 to 14.67 mgL^{-1}, 2 to 8 mgL^{-1}, and 40.43 to 107.63 mgL^{-1} respectively at different sites throughout the study period. The recorded TSS value ranged from 818.33 to 3,180 mgL^{-1}, TDS ranged from 90.67 to 726.67 mgL^{-1}. We observed that the minimum recorded TS (total solids) were 1,039.33 mgL^{-1}, where maximum TS were 3,897.33 mgL^{-1}. However, the turbidity of the Halda river water samples ranged from 40.09 to 355 NTU among the studied sites.

Determination of heavy metal concentrations

To explore the pollution level of Halda River water by inorganic pollutants, the heavy metal concentrations were

Table 1. At a Glance the Measured Value of Physicochemical Properties of the Water Samples of Halda River in February, May, and December 2019.

PARAMETERS	SAMPLING SITES															WHO STANDARD (2011)
	MOHARA WATER TREATMENT PLANT POINT			POINT OF KRISHNA KHAL			WEST SITE OF MODUNAGHAT BRIDGE POINT			EAST SITE OF MODUNAGHAT BRIDGE POINT			RAMDASH HAT POINT			
	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	
Temperature (ºC)	23.03	27.67	25.03	23.9	26.83	26.03	22.5	26.90	23.77	24	26.27	24.1	23.53	26.43	22.5	20–30 (Environmental Quality Standard [EQS], 1997)
pH	7.17	7.23	7.33	7.27	7.03	7.53	7.37	6.9	7.4	7.3	7.17	7.43	7.53	6.9	7.33	6.5–8.5
EC (μScm^{-1})	283.33	66.67	110	103.33	50	90	90	73.33	110	90	60	113.33	110	50	120	800–1,000
DO (mgL^{-1})	13.7	8.33	10.67	11.7	8	13.33	12.7	6	12	13	8.33	14	12.3	8.67	14.67	4–6
BOD (mgL^{-1})	8	3	4.34	7	2	4.3	6.7	4	5.33	6	3	3.2	3.3	4	5	5
COD (mgL^{-1})	105.5	98.03	107.63	66.1	62.83	76.69	85.8	70.29	79.89	66.6	56.43	69.23	69.8	52.16	40.43	10
TSS (mgL^{-1})	1,260	1,624	930.67	1,516.7	2,485.33	2,187.33	1,306.7	3,180	863.67	2,575.3	2,860	1,489.33	1,112	2,036	818.33	150*
TDS (mgL^{-1})	461.7	285.33	108.67	416.7	726.67	590.67	180	717.33	263.67	358.3	712.67	176.67	176.7	90.67	437.33	1,000*
TS (mgL^{-1})	1,721.67	1,903.33	1,039.33	1,933.33	3,212	2,778	1,486.67	3,897.33	1,127.33	2,933.67	3,572.67	1,750	1,288.67	2,126.67	1,255.67	.
Turbidity (NTU)	68.3	231	64.33	86.7	244.33	40.09	98.7	267.67	49.66	84.3	229.33	89	85.7	355	121.33	5–25

Note. EC = electrical conductivity; DO = dissolved oxygen; BOD = biological oxygen demand; COD = chemical oxygen demand; TSS = total suspended solid; TDS = total dissolved solid; TS = total solids; Feb = February; Dec = December.
*150 and 1000 mgL^{-1} is the Bangladesh Standard for TSS and TDS of inland surface water respectively.

determined and shown in Table 2.The results revealed that the average concentration of Pb, Cd, Cr, Ni at all study sites in the month of February, May, and December were below detection limit except at Ramdash Hat point in May, where the concentration of Pb ($6.765 \pm 0.244\,\mu gL^{-1}$) and Cr ($3.17 \pm 0.168\,\mu gL^{-1}$) was lower than the maximum standard limit 10 and $50\,\mu gL^{-1}$ respectively (WHO, 2011). Noticeable concentrations of Cu were recorded both at Mohara water treatment plant point ($4.622 \pm 0.080\,\mu gL^{-1}$) and Ramdash Hat point ($10.338 \pm 0.232\,\mu gL^{-1}$) in February but did not exceed the WHO permissible limit ($<40\,\mu gL^{-1}$) (WHO, 2011). Therefore, no pollution due to heavy metals was observed during the present investigation.

Microbiological assessment of water samples

In this study, a number of microbiological parameters were investigated to analyze the water quality of the Halda river and the results are shown in Table 3. The observed Total Bacterial Count (TBC) of water samples was found in the range from 7.2×10^3 to 3.83×10^5 CFU/mL. Moreover, the recorded Total Coliform (TC) and Fecal Coliform (FC) of the water samples of Halda River were in the range from 5.4×10^2 to 2.2×10^4/100 mL and 0.7×10^2 to 3.9×10^3/100 mL.

Identification and characterization of selected bacterial isolates

In this study, 25 bacterial colonies were isolated from water samples and based on their cultural and morphological characteristics 10 isolates (designated as HSA2, HSA4, HSB1, HSB2, HSC1, HSC3, HSD2, HSD3, HSE2, and HSE3) were randomly selected for identification. The isolates were taxonomically identified using 16S rRNA gene-based analysis (Table 4, Figure 2), morphological and biochemical characteristics. Results of 16S rRNA gene sequence of HSA2, HSB1, HSB2, HSC1, HSD2, HSD3, HSE2, and HSE3 strains showed maximum similarity to the genus *Bacillus* and HSA4 and HSC3 strains showed maximum similarity to the genus *Comamonas* and *Acinetobacter*, respectively. Phylogenetic relationship analysis of these isolates with the top hit strains from NCBI database also supports this (Figure 2). Identification by morphological, biochemical, physiological properties of these isolates (Supplemental Table 1 and 2) were also found consistent according to Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974).

According to NCBI database the 10 strains have been approved as novel strain viz. *Bacillus wiedmannii* strain HSA2, *Comamonas jiangduensis* strain HSA4, *Bacillus halotolerans* strain HSB1, *Bacillus safensis* strain HSB2, *Bacillus subtilis* strain HSC1, *Acinetobacter modestus* strain HSC3, *Bacillus subtilis* strain HABIBD2, *Bacillus marisflavi* strain HSD3, *Bacillus aerius* strainHSE2, and *Bacillus megaterium* strain HABIBE3 (NCBI accession numbers are mentioned in Table 4).

Table 2. Heavy Metal Profile of the Water Samples of Halda River in February, May, and December 2019.

HEAVY METALS (μgL^{-1})	SAMPLING SITES															WHO STANDARD (2011)
	MOHARA WATER TREATMENT PLANT			POINT OF KRISHNA KHAL			WEST SITE OF MODUNAGHAT BRIDGE			EAST SITE OF MODUNAGHAT BRIDGE			RAMDASH HAT			
	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	
Lead	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.765 ± 0.244	BDL	10
Cadmium	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	30
Chromium	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.173 ± 0.168	BDL	50
Nickel	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	70
Copper	4.622 ± 0.080	BDL	3.034 ± 0.222	BDL	3.147 ± 0.238	BDL	BDL	3.015 ± 0.155	BDL	BDL	4.775 ± 0.903	BDL	10.338 ± 0.232	6.091 ± 0.241	BDL	<40

Note. BDL = below detection limit; Feb = February; Dec = December.

Table 3. Microbiological Analysis of the Water Samples of Halda River in February, May, and December 2019.

PARAMETERS	SAMPLING SITES															
	MOHARA WATER TREATMENT PLANT			POINT OF KRISHNA KHAL			WEST SITE OF MODUNAGHAT BRIDGE			EAST SITE OF MODUNAGHAT BRIDGE			RAMDASH HAT			WHO STANDARD (2011)
	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	FEB	MAY	DEC	
Total bacterial count (CFU/mL)	2.79×10^4	2.91×10^5	1.11×10^5	1.08×10^4	1.76×10^5	1.9×10^4	7.2×10^3	2.5×10^5	4.07×10^4	2.50×10^4	1.12×10^5	2.8×10^4	7.5×10^3	3.83×10^5	8.67×10^3	-
Total coliform (TC)/100 ml	3.9×10^3	2.2×10^4	3.2×10^3	2.1×10^3	9.4×10^3	1.7×10^3	3.2×10^3	3.9×10^3	2.6×10^3	1.4×10^3	2.1×10^3	5.4×10^2	1.1×10^3	2.2×10^3	7.9×10^2	5×10^3
Fecal coliform (FC)/100 ml	9.4×10^2	3.9×10^3	4.9×10^2	3.4×10^2	4.5×10^2	3.8×10^2	5.4×10^2	7.0×10^2	4.3×10^2	1.1×10^2	2.4×10^2	0.7×10^2	1.4×10^2	2.6×10^2	1.1×10^2	4×10^2

Note. Feb = February; Dec = December.

Antibiotic susceptibility assay of the identified isolates

The results of antibiotic susceptibility assay are shown in Table 5. *Bacillus wiedmannii* strain HSA2 showed resistance against six antibiotics (multi-drug resistance) viz: cefalexin, ampicillin, amoxicillin, ceftazidime, ceftriaxone, and imipenem. *Comamonas jiangduensis* strain HSA4, *Bacillus halotolerans* strain HSB1, *Bacillus safensis* strain HSB2, *Bacillus subtilis* strain HSC1, *Bacillus subtilis* strain HABIBD2, *Bacillus maris-flavi* strain HSD3, and *Bacillus megaterium* strain HABIBE3 were found to form variable zone of clearance against all the antibiotics used in the current study. *Acinetobacter modestus* strain HSC3 was resistant against cefalexin, ampicillin, and sensitive to other antibiotics. *Bacillus aerius* strain HSE2 was also sensitive to all the antibiotics except ceftazidime.

Discussion

Now-a-days, water pollution is one of the greatest health problems in the world, especially in developing countries. Therefore, physicochemical and microbiological properties of surface water of the Halda River are considered as vital features of the water quality standards. According to the EQS (1997), the standard range of temperature of surface water is 20°C to 30°C. In this study, the temperature was found within the EQS standard limit. During spawning time, the temperature was also within the EQS standard limit in all sites (Table 1) (Ahmed et al., 2010; Hasan et al., 2019; Karim et al., 2019; Patra & Azadi, 1985)

On the other hand, the measured pH value was within permissible limit of surface water (Asian Development Bank [ADB], 1994; Ayres & Westcot, 1985; WHO, 2011). However, the results of the current study are in concurrence with other reports (Ahmed et al., 2010; Bhuyan & Bakar, 2017; Khan et al., 2020; Patra & Azadi, 1985).

During this study period, the average EC value was within the standard level of EC of most natural water (Department of Environment [DoE], 1997). The conductivity of most natural water generally ranges from about 50 to 1500 μScm^{-1} (Uddin et al., 2014). The WHO standard value is also reported as 1,000 μScm^{-1}(WHO, 1993b). Our results of EC were found (Table 1) within the permissible limit (Ahmed et al., 2010; Bhuyan & Bakar, 2017; Karim et al., 2019; Patra & Azadi, 1985). In addition the EC value was reported in Karnaphuli River (Dey et al., 2017) which is a matter of concern as the Karnaphuli River water intrudes into the Halda River.

According to WHO (1993a), EQS (1997), and others the standard value of DO in surface water is 4 to 6.5 mgL^{-1}(De Anil, 2005; DoE, 1997; Khan et al., 2020 Chen et al. (2012) reported that the DO concentrations of healthy water remain above 6.5 to 8 mgL^{-1} and this represents the good quality of river water. In the present investigation, the DO value in the spawning time was 6 to 8.67 mgL^{-1}, which shows the suitable DO concentration for spawning. The dissolved oxygen (DO) is

Table 4. Taxonomic Similarity Inferred From the Sequence Identity of Their 16S rRNA Genes. NCBI Accession Numbers are given in Parentheses.

ISOLATE NAME	NCBI BLAST RESULT (TOP HIT SP.)	PERCENT IDENTITY (%)	IDENTIFIED STRAINS (NCBI ACCESSION NO.)
HSA2	*Bacillus wiedmannii*	100.00	*Bacillus wiedmannii* strain HSA2 (MT102620)
HSA4	*Comamonasjiangduensis*	98.41	*Comamonas jiangduensis* strain HSA4 (MT102621)
HSB1	*Bacillus halotolerans*	99.74	*Bacillus halotolerans* strain HSB1 (MT102631)
HSB2	*Bacillus safensis*	99.67	*Bacillus safensis* strain HSB2 (MT102632)
HSC1	*Bacillus subtilis*	99.55	*Bacillus subtilis* srain HSC1 (MT102625)
HSC3	*Acinetobactermodestus*	98.07	*Acinetobacter modestus* strain HSC3 (MT102626)
HSD2	*Bacillus subtilis*	99.67	*Bacillus subtilis* strain HABIBD2 (MT102635)
HSD3	*Bacillus marisflavi*	100.00	*Bacillus marisflavi*strain HSD3 (MT102628)
HSE2	*Bacillus aerius*	99.46	*Bacillus aerius* strain HSE2 (MT102636)
HSE3	*Bacillus megaterium*	99.82	*Bacillus megaterium* strain HABIBE3 (MT102637)

an indication of available gaseous oxygen present in the water body, which is necessary for many forms of life. Considering the present study, it is showed that the solubility of oxygen in water decreases as temperature increases in May, and the colder water during February and December have the capability to hold higher concentrations of DO. The DO value found higher in February and December as sampling was done at the time of high tide and due to this high flow causing turbulence from opposite direction which increases DO level. Moreover, non-point source pollution due to heavy rain fall occurs during the month of May in Bangladesh. As a results rainfall picking up natural or anthropogenic (human-made) pollutants and depositing them into water resources that reduce the DO value in the month of May. On the other hand, the BOD level of the Halda River water represents the river is moderately polluted (Connor, 2016). High BOD was found such a point where canals like Krishna Khal, Madari Khal (near Ramdash Hat point) connect to the Halda River and especially at the sites of Mohara water treatment plant sampling point, where Halda connects to the polluted Karnaphuli River and water intrudes into Halda River during high tide (Ahmed et al., 2010; Islam et al., 2017). Comparing with previous findings, it showed that the BOD of the Halda River water is increasing day by day.

However, COD of water samples showed great variance among the sampling sites. In the present study, the recorded COD values are highly deviated from the WHO standard value (10 mgL^{-1}) (Table 1). The recorded higher COD value indicates the breaking down of organic and inorganic material entering the river incessantly. The high COD value was reported by Uddin et al. (2014) in the Jamuna River, Dey et al. (2017) in the Karnaphuli River and Karim et al. (2019) in the Halda River. Studies revealed that Halda River is being polluted with organic substances which are showing increased COD value (Ahmed et al., 2010; Bhuyan & Bakar, 2017) which are close to the values found in the present study.

In the study period, average TSS of water samples at different points was measured (Table 1) which is mostly deviated from the Bangladesh standard for TSS (150 mgL^{-1}) of inland water. In comparison with the previous studies, it can be noted that suspended solids in the Halda River water are increasing day by day (Ahmed et al., 2010; Islam et al., 2016). The suspended solids from various sources including sand lifting, industrial and domestic discharges, etc., causing the river to be polluted (Hussain, 2020; Islam et al., 2017).

In addition, the recorded Total Dissolved Solids (TDS) in the study period were within the Bangladesh Standard for TDS of inland surface water (1,000 mgL^{-1}). But there are concern matters about the TDS value of the river water which is gradually increasing day by day (Ahmed et al., 2010). Due to the increased amount of suspended solids, the Total Solids (TS) of the river was also higher than ever before and the average TS value of the Halda River water in the sampling months at different sampling points ranged from 1,590.07 to 2,952.4 mgL^{-1}, which is a sign of pollution (Islam et al., 2016; Sarwar et al., 2010). Moreover, the present study revealed that the average results of turbidity of water samples indicates that the Halda River water was highly turbid than the permissible limit (5 –25 NTU) (Karim et al., 2019).

Based on the present investigation, there was no pollution found in the Halda River water with lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu). Therefore, almost in all sampling sites on the Halda River water, the concentration of heavy metals were attributed to the WHO permissible limit (WHO, 2011) (Table 2). Ahmed et al. (2010) studied the Halda River water did not find any toxic heavy metal except manganese. Similarly, Islam et al. (2016) also reported that there was no pollution from Cd, Cr, Ni, and Cu in Halda River water.

Before the present work, only a few groups described the presence of microbial contamination of Halda River water. In this study, higher count of TBC was recorded in May during

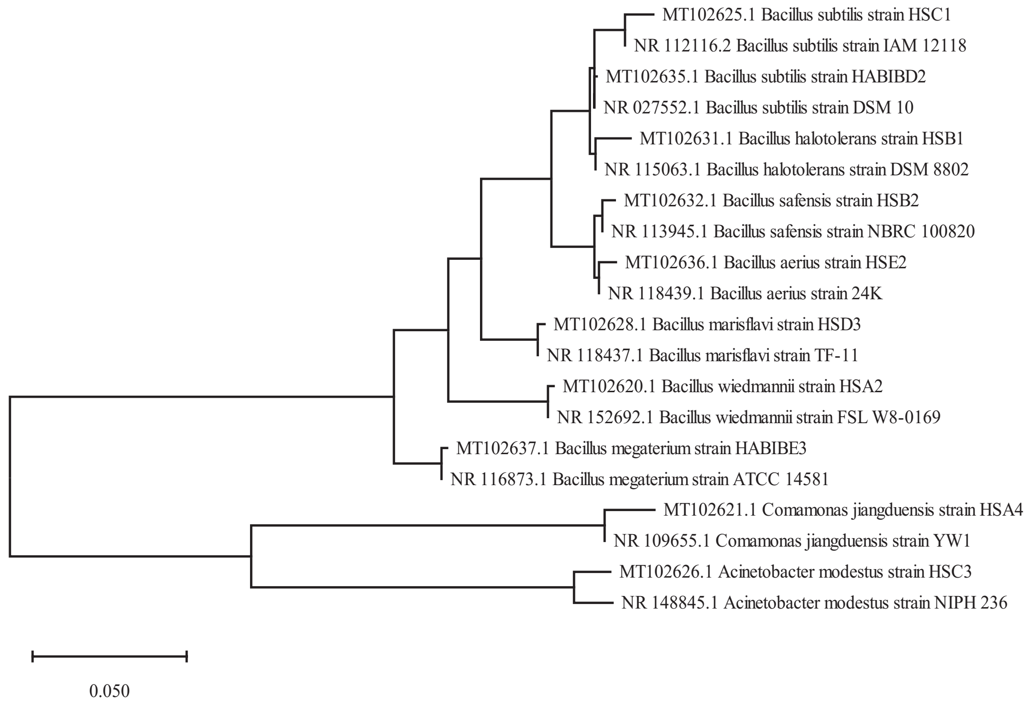

Figure 2. Evolutionary relationship analysis: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (−6107.99) is shown. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 1,562 positions in the final dataset. Evolutionary analyses were conducted in MEGA11(Tamura et al., 2021).

the spawning season. This may be due to excessive nutrient run-off or microbes washing off from land with rainwater flow along with wastes containing bacterial contamination from different sources, such as unplanned industrialization near the river, local market on the bank of river, domestic discharge, connectivity of canals, flowing of sea water through Karnaphuli, etc. (Ahmed et al., 2019; Illius, 2021; Islam et al., 2016; Safiur Rahman et al., 2021). Moreover, previous studies reported that higher bacterial count was observed in different river water of Bangladesh (Real et al., 2017; Saha et al., 2009; Tuhi et al., 2017).

According to WHO standard, the maximum permissible limit of TC is 5×10^3/100 mL (WHO, 1993a). The Total Coliform count in the Halda water samples mostly were within this limit except at Krishna Khal point and Mohara water treatment plant point (Table 3) in May, which was highly polluted with Coliform where the Halda connects to the Karnaphuli River. Therefore, this pollution may be from Karnaphuli River which was reported with high count of Total Coliform (Dey et al., 2017) as water enters into the Halda River during high tide (Islam et al., 2016). However, among the sampling sites, water samples from Mohara water treatment plant were found highly polluted by fecal coliform than other sampling sites. Pollution at Mohara water treatment plant point may be from Karnaphuli River (Dey et al., 2017) as sampling was done at the time of high tide and water from Karnaphuli River intruded into Halda River (Islam et al., 2016). Higher count of fecal coliform was also found in the west site of Modunaghat Bridge.

Ten bacteria were identified from the Halda River water (Table 4). The occurrence of *Bacillus, Acinetobacter*, and *Comamonas* during this study in water samples is of primary importance because the river water may be contaminated from different sources. The contamination may be from the local market, Modunaghat Bazar, domestic and discharges from different sources through canals that are connected to Halda River. However, *Bacillus halotolerans* strain HSB1, *Bacillus safensis* strain HSB2, *Bacillus subtilis* strain HSC1, *Bacillus marisflavi* strain HSD3, and *Bacillus megaterium* strain HABIBE3 in this study are highly salt tolerant (Supplemental Table 2), possibly they are from sea water or tannery wastes. Oyelakin et al. (2016) isolated *Comamonas jiangduensis* from the fish *Brycinus longipinnis* at Eggua Station in Yewa River, Nigeria as a potential pathogen. However, all the identified strains were tested for their antibiotic sensitivity against commercially available disk of antibiotics. It is a matter of concern that *Bacillus wiedmannii*strain HSA2 showed resistance against a number of commercially available antibiotics. Previously reported that *Bacillus wiedmannii* is a psychrotolerant and have strong cytotoxic effect (Miller et al., 2016). A recent study also reported the toxic effect of this bacterium (Zhao et al., 2019).

Table 5. Antibiotic Susceptibility Assay for the Identified Isolates Against Some Commercially Available Antibiotics.

NO OF ISOLATES (IDENTIFIED SPECIES)	ANTIBIOTICS ZONE OF INHIBITION (DIAMETER IN MM)									
	CN	AMP	AML	CIP	CAZ	CTR	AK	C	MEM	IPM
HSA2 (*B. wiedmannii*)	0	0	0	32	0	0	23	21	22	0
HAS4 (*C. jiangduensis)*	10	25	33	25	20	32	24	37	42	30
HSB1 (*B. halotolerans)*	35	29	32	42	29	40	25	34	44	38
HSB2 (*B. safensis*)	17	25	28	36	8	10	29	25	32	25
HSC1 (*B. subtilis)*	33	40	44	42	12	33	35	37	40	33
HSC3 (*A. modestus*)	0	0	19	44	11	18	30	29	18	24
HSD2 (*B. subtilis*)	28	37	26	40	16	34	29	36	39	30
HSD3 (*B. marisflavi)*	11	20	22	30	20	34	30	30	38	15
HSE2 (*B. aerius)*	24	31	34	36	0	18	29	26	37	31
HSE3 (*B. megaterium)*	28	23	26	32	29	30	28	32	35	31

Note. Each data is representative of three independent measurement of antibiotic sensitivity test. CN = cefalexin 30 µg; AMP = ampicillin 2 µg; AML = amoxicillin 30 µg; CIP = ciprofloxacin 5 µg; CAZ = ceftazidime 30 µg; CTR = ceftriaxone 30 µg; AK = amikacin 30 µg; C = chloramphenicol 30 µg; MEM = meropenem 10 µg; IPM = imipenem 10 µg.

Conclusion

This study can be concluded that the Halda River water is being polluted from the intrusion of Karnaphuli River water and discharges from various industrial sources. This can significantly affect the growth, distribution and physiology of fish and other lives of this unique natural carps breeding gound of Bangladesh. Considering the physicochemical pollution level, occurance of high microbial load and pathogenic bacteria indicates that the Halda River water is becoming unsafe for drinking and domestic purposes. To minimize the contamination and pollution level of Halda River, a great effort from different authorities such as locals, farmers, agriculturists, municipal, industrialists, ministries of government is needed. This can be achieved by the proper treatment and discharge of agricultural, industrial, sewage wastes, wastes from mills or factories. So, proper management and planning for water quality monitoring is necessary to maintain a healthy ecosystem of Halda River. Moreover, regular monitoring and evaluation of water quality of the Halda River are also recommended.

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Author Contributions

Md. Towhid Hossain and Jannatul Ferdouse contributed to conception and design and supervised the study; Md. Habibur Rahman carried out laboratory experiments and generated and analyzed the data; A. K. M Atique Ullah performed heavy metal analysis. Md. Habibur Rahman and Jannatul Ferdouse wrote and prepared the manuscript; Md. Towhid Hossain reviewed the manuscript; all authors read and approved the final manuscript.

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All authors approve the research, and no human or animals were involved in this study.

ORCID iD

Md Habibur Rahman https://orcid.org/0000-0001-6942-0888

Supplemental Material

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