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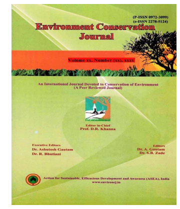

Assessment of water quality using different physicochemical and biological parameters: a case study of Buddha nallah, Punjab, India

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ABSTRACT

For the assessment of physicochemical and microbiological quality of Buddha Nallah the water samples were drawn from 7 different sites and analysed during winter (December 2020) and summer (May 2021) for most probable number, heterotrophic plate count, total coliform, fecal coliform, indicator, emerging pathogens and physicochemical parameters. A strong correlation was found among the indicator organisms (r= 0.504-0.898), while relatively weak or no correlation was found between indicator and emerging pathogens. Moreover, the correlation between indicator and emerging pathogens was found to be heavily dependent on physicochemical parameters. Cluster analysis successfully classified the different polluted sites based on physicochemical and microbiological parameters. The water quality index (WQI) score of all sites was found between 0-25 indicating poor water quality and emergency treatment is required for reuse. Based on present study results, it has been concluded that water of study area is highly polluted and pose serious health risk concerns due to presence of fecal and emerging pathogens in samples.

Introduction

Water pollution is a global threat to both natural diversity and human health. With increase in urbanization / industrialization and to achieve global food security, the use of chemical pesticides, mining and other anthropogenic processes has been increased. These practices result in the deterioration of water bodies and increases morbidity rate (Bhutiani *et al.,* 2021a; Ruhela *et al.,* 2022). Annually about 37.7 million Indian population got affected by water-related illness resulting in $600 million economic cost. It has been estimated that 70 % of surface water in India is unfit for human consumption. Every day about 40 million liters of wastewater enters surface waters, groundwater and other water bodies (Bhutiani *et al.,* 2021b). Water contamination from human waste, agriculture runoffs, and industrial effluent discharges is related to the release of toxic compounds that can stimulate the rapid and excessive growth of microbial pathogens. The risk of contamination increases when waterborne pathogens and nutrients, such as nitrates, phosphorous and nitrogen, are transported from residential, agricultural and industrial areas to natural streams like ponds, lakes, rivers, sea and finally to ocean, causing serious disruption in aquatic biodiversity (Sinha *et al.,* 2016; Ruhela *et al.,* 2021). Assessment of water quality using physicochemical and biological parameters have been studied to protect the biodiversity and to rejuvenate the water resources reported in literature (Jindal and Sharma, 2011; Mavukkandy *et al.,* 2014; Bhutiani *et al.,* 2018; Kaur *et al.,* 2021; Das *et al.,* 2021). Total Coliform (TC) and Fecal Coliform (FC) have been used for assessment of water quality and emergence of waterborne pathogens in water. According to the World Health

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Organization (WHO) and Bureau of Indian Standards (BIS), *Escherichia coli* is widely used as indicator of fecal contamination, as it is highly tolerant to environmental changes and disinfection than viruses, bacteriophages etc. However some reports have shown a weak relation between coliform and waterborne pathogens that leads to uncertainty in quantifying the exposure (Goh *et al.,* 2019).

Ludhiana is a district of the Punjab, India with catchment area 159 kilometer square. It is a fast growing industrial hub of northern India and referred as Indian Manchester by BBC in 2014 (Anonymous, 2014). Until the early 90's, the residents of Ludhiana uses "Buddha dariya" water for drinking and domestic purposes. But due to ongoing continuous discharge of wastewater like industrial effluents, sewage disposal and agricultural runoffs water gets extremely deteriorated and name got changed to "Buddha Nallah" by local people. This polluted water is being used by the farmers for irrigation purposes in this area and cause high metal toxicity in soil which leads to high human health risks (Kaur *et al.,*2021). According to PGIMER Chandigarh and Punjab Pollution Control Board, the villages near the Nallah contain more amounts of calcium, magnesium, mercury, fluoride and heptachlor than the permissible limits in tap and ground water. Several projects have been involved in rejuvenation of water quality of Buddha Nallah like on April 2011 Indian Ministry for Environmental and Forests installed 'In-Situ Bioremediation project' on Buddha Nallah. In 2021, Punjab Government launches 840 crore Buddha Nallah rejuvenation project for domestic wastewater management.

Keeping all these points, this study is focused on assessment of water quality of Buddha Nallah, Ludhiana, India during winter (December 2020) and summer (May 2021) season. The present study is being undertaken with the following objectives: (1) to generate a database of physicochemical and microbiological quality of Buddha Nallah water with season variability and to correlate with pathogenicity; (2) to assess the emergence of indicator and emerging pathogens in water with seasonal 103 variability; (3) to identify the correlation between physicochemical, biological and indicator/emerging pathogens.

Material and Methods

Sampling sites and processing

The Buddha Nallah or Buddha dariya is a natural seasonal water stream that runs through the Malwa region of Punjab, India. It originates from Koom Kalan, Punjab (76°06′55.30′′E, 30°92′07.21′′N) and passes through Ludhiana city, where it ultimately drains into the Sutlej River near village Walipur (75°62′69.02′′E, 30°97′49.74′′N), District Ludhiana, Punjab, India. Sample sites of Buddha Nallah were selected to represent possible pollution sources for industrial effluent discharges, agricultural runoffs, dairy and domestic wastes. Water samples were collected from 7 sites of Buddha Nallah (Table 1, Figure 1) during the winter and summer. A total of 14 water samples were collected during winter (December 2020) and summer (May 2021) from 7 sites of Buddha Nallah, Ludhiana, India. The water samples were collected from approximately 20cms depth of the water surface in sterile bottles (1L capacity) under aseptic conditions to avoid other bacterial contamination from different locations. The sample bottles were tightly capped and labelled with the site, time, and date of collection and stored at 4°C during transit to the laboratory. Water sampling, handling, preservation, and processing were done by following standard protocols (WHO, 2008; USEPA, 2012).

Physicochemical analyses

Water samples were analysed for pH, water temperature, electrical conductivity (EC), nitrates, sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}) as standard procedures of American Public Health Association (APHA 2012). Calcium, magnesium, chloride, and nitrate were determined using the titration method, whereas sodium and potassium were analysed through flame spectrometry. Biological oxygen demand (BOD) and dissolved oxygen (DO) were estimated using the Winkler titration method (Winkler 1888) with azide modification followed by five days of incubation in dark conditions at 20°C under aerobic conditions (for BOD samples).

Total heterotrophic and coliform count

Total Heterotrophic plate count (THPC) includes all the micro-organisms capable of growing in nutrient-rich media.

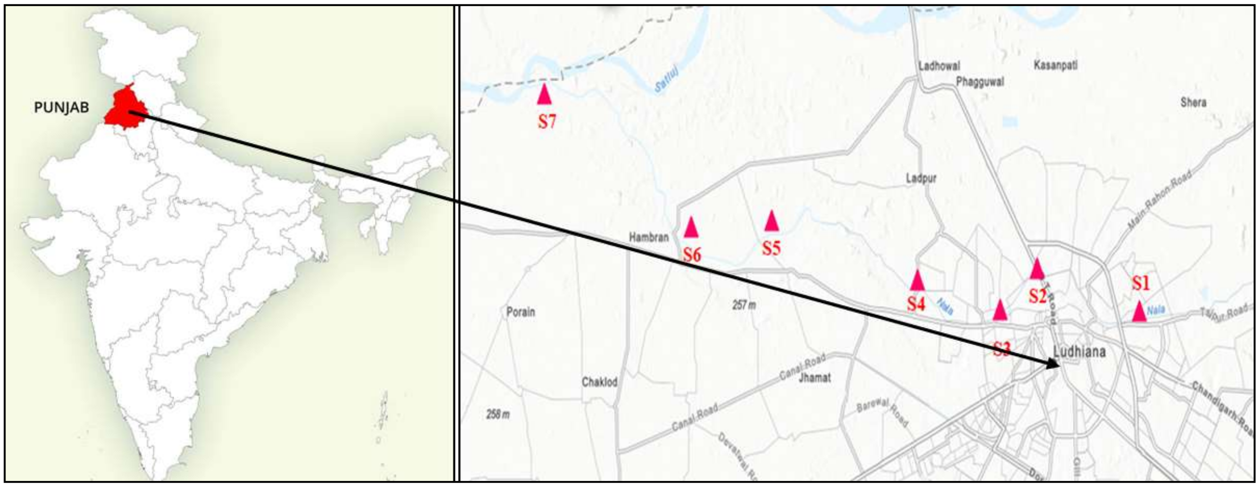

Figure 1: Study area map indicating (A) Punjab location in India map (B) Labeled sampling sites (S1 to S7) of Buddha nallah, Ludhiana, Punjab, India. (*Source (A) from Google images (B) assessed from USEPA (US environment protection agency) site through DWMAP* https://www.epa.gov/sourcewaterprotection*.)*

Table1: Description of sampling location and pollution sources in Buddha nallah, Ludhiana, India

Sampling sites	Description	Co-ordinates		Waste sources
		Longitude	Latitude	
S1	Tajpur road, near Haibowal Kalan, Ludhiana, India	75°87′70.45″ E	30°91′72.29″ N	Domestic, agricultural, dairy and textile waste effluent
S2	GT Road Ladhowal, New Kitchulu Nagar, Ludhiana, India	75°79′20.31″ E	30°92′53.33″ N	Domestic, agricultural and dairy
S3	Village Baran Hara, Distt. Ludhiana, India	75°76′94.73″ E	30°93′76.93″ N	Domestic and dairy
S4	Village Talwara, Distt. Ludhiana, India	75°74′90.01″ E	30°94′22.44″ N	Domestic, dairy and agricultural
S5	Near Malakpur-Nurpur Bet Road, Distt. Ludhiana, India	75°72′54.39″ E	30°93′71.08″ N	Domestic and paper mills effluent
S6	Near Hambran Laddowal Road, Distt. Ludhiana, India	75°68′93.46″ E	30°93′94.53″ N	Domestic, agricultural and paper mills effluent
S7	Near Village Walipur Kalan where Buddha nallah emerges River Sutlej	75°65′11.87″ E	30°97′28.52″ N	Domestic and agricultural

All water samples were analysed for bacterial total heterotrophic plate count consisting of the mixing of aliquots of 1-ml water with nutrient agar (Hi-media) thoroughly and incubation at 37ºC for 24-48h to encourage the growth of bacteria associated with the human fecal flora. The number of bacteria were enumerated and expressed in $\log_{10}$ colony-forming unit per ml ($\log_{10}$ CFU/ml). The presence of coliform in water samples were confirmed through the multiple fermentation tube technique as a most probable number (MPN) index. The MPN tests are statistical methods based on probability dispersion analysis (APHA 1998, APHA 1971). Analysis of total coliforms (TC) and fecal coliform (FC) was carried out by using the membrane filtration method as described Koster *et al*., (2003).

The colonies of bacteria were enumerated and expressed in a $\log_{10}$ colony forming unit per ml ($\log_{10}$ CFU/ml).

Isolation of indicator and emerging pathogens

The enriched samples from positive MPN tubes were cultured for the presence of *E.coli*, Urinary tract infection (UTI) bacteria, *Vibrio spp*., *Salmonella spp*., *Shigella spp*., *Aeromonas spp*., *Listeria spp*., and *Staphylococcus spp*. by following the standard procedures (Koneman *et al.,* 1983). The samples were inoculated on selective media procured from Himedia Pvt. Ltd; Eosin Methyl Blue gar (EMB agar), Thiosulfate Citrate Bile salts Sucrose agar (TCBS agar), Salmonella Shigella agar (SS agar), Aeromonas selective agar supplement with ampicillin, Listeria selective agar with Listeria supplement, Baird parker agar supplement with 1% potassium tellurite followed by incubation at 37˚C for 24-48h. Bacterial pathogens were identified based on phenotypic and biochemical characterization that was further confirmed by using Bergey's Manual of Determinative Bacteriology. The single colony of each pathogen selected from the agar plate and streaked onto freshly prepared nutrient agar slants were stored in trypticase soy broth containing 30% glycerol stock solution at -20˚C.

Water quality index (WQI)

The water quality index (WQI) method is used to evaluate the overall water quality based on physico-chemical and biological parameters for multiple usage purposes. Based on the calculated water quality index (WQI), water samples were categorized into different categories. Lesser the WQI score; more the water pollution level and greater risk to health. The WQI calculated using the following equation (VEA 2011):

$$\boldsymbol{WQI} = \frac{\boldsymbol{WQI_{pH}}}{\mathbf{100}}\left[\frac{\mathbf{1}}{\mathbf{5}}\sum_{a=1}^{5}\boldsymbol{WQI_a} \times \frac{\mathbf{1}}{\mathbf{2}}\sum_{b=1}^{2}\boldsymbol{WQI_b} \times \boldsymbol{WQI_c}\right]\frac{\mathbf{1}}{\mathbf{2}}$$

Where WQI_{pH} represents pH; WQI_a for chemical parameters (BOD, DO, nitrates, Na, K, and chlorides); WQI_b for physical parameters (EC and total hardness) and WQI_c for biological parameters (coliform count).

Statistical analysis

All the experiments were completed in triplicate and the data were presented as mean± standard deviation (SD). The Pearson method was selected for the correlation analysis to examine if there was a significant difference between the distributions of microbial population and physicochemical parameters with seasonal variability at different sites. Cluster analysis was used to classify the sampling sites based on physicochemical and microbial quality of water during the winter-summer season. Data was analysed using SPSS software (version 16.0, SPSS Inc.).

Results and Discussion

Water samples were collected along the course of Buddha Nallah and analysed for physicochemical parameters (pH, temperature, EC, BOD, DO, Ca, Mg, Na, K, Nitrate and Chlorides) and microbiological qualities (MPN index, Heterotrophic plate count, Total coliforms, Fecal coliform, indicator and emerging pathogens) during the winter (December 2020) and summer (May 2021) season before second COVID lockdown. So, the seasonal variation should be observed and water quality can be assessed.

Seasonal variation in physicochemical analyses of Buddha Nallah

All physicochemical parameters analysed during different seasons were presented in Table 2. The water samples analysis of Buddha Nallah revealed that in winter, pH of different sites varied from 6.1 to 6.96 and 6.09 to 7.18 during summer season. As high or low pH in water is an indicator of presence of chemical or heavy metal pollution. The maximum value of pH is observed in summer and minimum in winter that attributed to increase or decrease in inorganic and organic pollutants. pH values for sites S1, S3, S6 and S7 were found to be acidic in nature and below the permissible limits (6.5-8.5) prescribed by WHO (2011). Acidic nature of water is an indication of contamination and unsafe for drinking purposes. Matta *et al.*(2017) recorded variation in pH in Ganga River with season variability due to discharge of industrial waste.Water temperature is one of most important characteristics of an aquatic system, as it directly affects the dissolved oxygen (DO) levels, chemical and biological processes in water. With increase in temperature, the solubility of oxygen decreases in water. In Buddha Nallah, water temperature ranged between 26.3˚C (S1) to 28.2˚C (S6) during winter and 33.8˚C (S1) to 36.9˚C (S6) in summer season.

Table 2: Seasonal variation of physicochemical characteristics of Buddha Nallah water samples

Parameters*	S1	S2	S3	S4	S5	S6	S7
Winter Season							
pH	6.21±0.01	6.80±0.02	6.36±0.14	6.96±0.04	6.27±0.06	6.10±0.10	6.25±0.04
Temp (˚C)	26.3±0.20	27.8±0.25	26.6±0.20	27.3±0.80	26.8±0.65	28.2±0.40	27.3±0.25
EC (µS/cm)	3214±4.00	3554±4.00	3426±5.50	2685±2.00	2542±2.00	2274±4.00	1532±1.50
BOD (mg/L)	229.1±1.60	356.1±2.10	122.8±2.75	107.2±2.10	244.4±1.80	56±1.00	76±1.75
DO (mg/L)	BDL	1.31±0.11	BDL	BDL	0.66±0.03	0.98±0.04	4.68±0.40
Nitrate (mg/L)	21.1±1.05	19.4±0.10	16.7±0.95	20.0±0.50	18.2±0.95	11.9±0.40	12.2±0.20
Ca+Mg (mg/L)	241.7±1.70	140.4±2.68	134.9±1.19	346.3±5.07	234.5±1.79	148.8±3.58	215.9±3.36
Na^+ (mg/L)	306.6±4.55	389.2±0.95	276.5±2.00	337.7±5.15	274.2±3.00	322.3±2.95	276.3±0.90
K^+ (mg/L)	27.8±0.55	24.2±1.15	24.8±0.20	23.8±0.35	21.9±0.65	27.9±0.65	23.3±0.90
Choride (mg/L)	208.2±6.75	203.3±4.80	210.1±0.90	130.6±1.65	163±4.00	236±9.00	194.6±3.60
Summer season							
pH	6.03±0.00	7.17±0.01	6.45±0.10	7.18±0.02	6.59±0.03	6.09±0.01	6.15±0.02
Temp (˚C)	33.8±0.70	35.9±0.10	34.7±0.30	35.5±0.55	35.8±0.75	36.9±0.15	35.8±0.25
EC (µS/cm)	3911±0.50	4083±1.00	3932±1.50	3079±1.00	2864±3.50	2239±1.50	1823±3.00
BOD (mg/L)	370.1±0.75	422±0.00	257.6±3.60	213.4±1.20	247±1.00	82.2±2.00	101.8±1.50
DO (mg/L)	BDL	0.81±0.03	BDL	BDL	BDL	BDL	2.27±0.31
Nitrate (mg/L)	34.2±0.05	22.7±0.15	19.5±0.20	26.2±0.10	20.6±0.05	22.1±0.04	14.1±0.03
Ca+Mg (mg/L)	310.5±1.53	235.0±3.16	164.9±3.73	398.3±3.44	258.6±1.72	186.1±2.30	278.3±2.44
Na^+ (mg/L)	341.5±0.55	411±0.80	317.7±20.7	362.5±3.70	313.4±2.60	381.3±1.05	316.9±4.15
K^+ (mg/L)	27.0±0.10	28.9±0.25	34.8±0.18	35.0±0.26	31.6±0.18	34.1±0.60	32.9±0.13
Choride (mg/L)	273.2±1.75	257.3±0.80	219.7±0.25	155.6±3.35	173±4.00	261±6.00	206±5.00

Temp: water temperature; EC: electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K:potassium

The fluctuation in temperature at different sites might be due to discharge of cooling water and heated industrial effluent into water. Similar results were observed by Bhatia *et al.,*(2018), in Buddha Nallah during the study period of 2014.Increase in ion concentration or dissolved solids in water enhance its electrical conductivity (EC). According to WHO (2011) standards, EC value should not exceed 1000µS/cm. In current study, EC value varied from 1532µS/cm (S7) to 3554µS/cm (S2) in winter and 1823µS/cm (S7) to 4083µS/cm (S2) in summer season. Electrical conductivity (EC) was observed to be relatively high in upstream as compared with the downstream sites. These results clearly indicate that water in the present study area contain high amount of dissolved solids and is highly polluted with industrial and domestic discharges (Jindal and Sharma 2011).

Dissolved oxygen (DO) is the most well-established parameter of water quality assessment and essential for the survival for all aquatic life. Moreover, oxygen affects a vast number of water indicators, not only biochemical but also aesthetic ones like odour, clarity and taste. The water samples analyses of Buddha Nallah seem to indicate low level of dissolved oxygen (DO) as compared to standards given by WHO (2011) which ranged from 4-6mg/L. In Buddha Nallah, the DO found to be more in downstream as compared to upstream sites. Majority of sites were observed to have DO below detection level (BDL) during both winter and summer season. This finding revealed that the pollutants in the water consumed a large amount of dissolved oxygen. Biological oxygen demand (BOD) is used as approximate measure of the amount of biochemically degradable organic matter present in sample. Laboratory analyses indicates BOD value varied to 56mg/L (S1)-356mg/L (S2) during winter and 82.2mg/L (S1)- 422mg/L (S2) in summer season. The increase in BOD values in summer season can be due to high microbial activities while decomposing organic matter under aerobic conditions (Yisa and Tijani 2010). Similar results reported by Mena-Rivera *et al.* (2017) in Burio river of Costa rica and Sharma *et al* . (2020) in Yamuna river of India.

Nitrate is oxidized form of dissolved nitrogen and required as main source of nitrogen for aquatic plants. However, in high amount nitrate can be toxic and leads to eutrophication in water bodies. In India, rising nitrate level in water resources is one of most challenging and growing water problems. Irrigation water containing fertilizers is a common source of nitrate contamination. In Buddha Nallah, nitrate content varied from 12.2mg/L (S2) to 21.1mg/L (S1) in winter and 14.1mg/L (S7) to 34.2 mg/L (S1) in summer season. The nitrate content in both seasons was found to be below the permissible limit of 50mg/L as prescribed by WHO (2011) and BIS (2012).The degree of hardness in water is expressed in the terms of calcium (Ca) and magnesium (Mg) content. According to WHO (2011), the permissible limit for Ca and Mg content in water should not exceed above 500mg/L. The concentration of Ca and Mg in Buddha Nallah at different sites during winter and summer season was found to be under prescribed values. Kaur *et al.*(2021) also observed calcium and magnesium contents present in Buddha Nallah within the permissible limits.

Sodium (Na^+) and potassium (K^+) are essential elements for human health. High amount of these elements in drinking water could be a concern for human health. In present study, results showed the presence of high amount of sodium (Na^+) and potassium (K^+) in water samples of both winter and summer season, particularly due to high amount of industrial effluent, domestic waste and agriculture runoff discharges. Chlorides occur in surface and groundwater from anthropogenic processes such as agricultural runoffs, industrial effluent, irrigation drainage and animal feeds. The high amount of chlorides in water increases electrical conductivity (EC) and led to corrosion of metal pipes. All the water samples contain chlorides within permissible limits (WHO 2011) except sites S1, S2 and S6 during the summer season that indicates the human health risks.

Seasonal variation in microbiological quality of Buddha Nallah

The results were analysed seasonally for the portability of water samples based on MPN index. It has been observed that in both seasons winter and summer all the samples were found to be non-potable, attributed to environment factors and industrial waste that favour the survival rate of microorganisms. Total Heterotrophic plate count (THPC) includes the natural micro-biota of water and also organisms derived from different pollutant sources e.g. industrial effluent, sewage mixing and agricultural runoffs. The maximum microbial load in water samples was recorded in summer as compared to winter season as shown in Fig2a.The highest log value of microbial count, winter and summer was recorded as 8.46±0.01 (S2) and 8.59±0.02 (S5), respectively. The variation in microbial count at sites has been attributed to the change in temperature, availability of nutrient and extent of pollution. Prasad *et al.*(2015) reported variation in microbial count from 1.0 to 3.2×10^{3} along east coast of India.

Coliform are often referred to as 'indicator organisms' because it indicate the presence of faecal contamination and enteric pathogens in the water system. The maximum total coliforms and faecal coliform count for water samples were found in summer as compared to winter season (Fig 2b, 2c). All samples were highly contaminated with the coliform and indicate the presence of water-borne pathogens in water. Maximum log value of Total coliform (TC) and Faecal coliform (FC) were ranged between 7.41±0.01 (S5) and 5.41±0.01 (S5), respectively during summer. In winter maximum log value of Total Coliform and Faecal Coliform was recorded as 7.16±0.02 (S7) and 5.15±0.00 (S7), respectively. The results revealed that water of Buddha Nallah for both seasons were equally contaminated with coliform organisms but counts were significantly higher in summer season. The high load of coliform attributed to the heavily populated area along Buddh Nallah and mixing of domestic/sewage wastewater to water.

Seasonal occurrence of indicator and emerging pathogens

According to World Health Organization (WHO) and Bureau of Indian Standards (BIS) standards, no water intended for human consumption shall contain *Escherichia coli* in 100ml of water sample. *E.coli* is an indicator of presence of the faecal contamination and act as surrogate microbe for water quality assessment. The samples were analysed for the occurrence of *E.coli* and relative abundance was recorded during winter and summer season with respect to temperature fluctuation (Fig 3).It has been observed that the incidences of indicator organisms have been detected in all samples. The maximum relative abundance of *E.coli* was 29.3% in winter and 29.1% in summer. The results suggested that seasonal variation doesn't affect the prevalence of *E.coli* in Buddha Nallah. In recent years emerging pathogens have arisen as a major public health concern. Emerging pathogens include urinary infection bacteria (UTI), *Vibrio cholera,V. parahaemolyticus, Aeromonas hydrophilia, Salmonella entrica, Shigella flexneri., Listeria monocytogenes and Staphylococcus aureus.* In present study, all emerging pathogens shared more than 1% relative abundance in different sites of Buddha nallah are presented in Fig 3.The results from Figure 3 shows that UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica,Listeria monocytogenes and Staphylococcus aureus* were detected in the both seasons, while the distribution *Shigella flexneri* varied greatly. In summer, there were 6 dominant emerging pathogens detected in the water samples. The maximum relative abundance of UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica,Listeria monocytogenes and Staphylococcus aureus* were found to be 31.1%, 14.1%, 12.4%, 13.6%, 14% and 11.2%, respectively. None of the samples were contaminated with *S. flexneri* during the summer season. In winter season, 7 emerging pathogens were analyzed in water samples. There was no significant difference in the pathogen abundance for the two seasons except for the *S. flexneri* which had significantly higher abundance in winter season. The maximum abundance of UTI bacteria, *Vibrio cholera, Aeromonas hydrophilia, Salmonella entrica, Shigella flexneri, Listeria monocytogenes and Staphylococcus aureus* in winter was recorded as 31.9%, 15.1%, 13%, 12.9%, 6.14%, 14.8% and 11.5%, respectively.

Correlation between microbial indicators, pathogens and physicochemical parameters

Correlation analysis was conducted with Pearson coefficient method. The correlation examined (i) between physiochemical parameters (Table 3); (ii) between microbial indicators/pathogens (Table 4); and (iii) between physicochemical parameters and indicators/pathogens (Table 5) in both seasons. This study found significant positive correlation among the physicochemical parameters; pH, EC, BOD, nitrate and temperature in both seasons. Wherein, with increase in EC and temperature, there is significant reduction in BOD and nitrate, respectively. Among the indicator organisms,

Table 3: Pearson correlation between physicochemical parameters

Winter season										
	pH	Temp	EC	BOD	DO	Nitrate	Ca+Mg	Na^+	K^+	Chloride
pH	1									
Temp	0.068	1								
EC	0.503*	0.549**	1							
BOD	0.694**	0.584**	0.773**	1						
DO	0.164	0.169	-0.632**	-0.270	1					
Nitrate	0.185	-0.439*	0.710**	0.601**	-0.680**	1				
Ca+Mg	-0.625**	-0.224	-0.259	-0.341	-0.264	0.417	1			
Na^+	0.432	0.476*	0.440*	0.413	-0.302	0.343	-0.062	1		
K^+	-0.214	-0.012	0.174	0.237	-0.410	-0.056	-0.254	0.060	1	
Chloride	0.302	0.191	0.098	0.320	0.209	-0.473*	0.841**	0.002	0.638**	1
Summer season										
pH	1									
Temp	-0.016	1								
EC	0.523*	0.567**	1							
BOD	0.513*	0.555**	0.852**	1						
DO	-0.252	0.183	-0.479*	-0.236	1					
Nitrate	-0.342	-0.553**	0.555*	0.490*	-0.304	1				
Ca+Mg	-0.340	-0.315	-0.037	0.023	0.017	0.672**	1			
Na^+	0.329	0.029	0.568**	0.533*	0.036	0.103	0.277	1		
K^+	0.328	0.380	-0.467*	-0.847**	-0.042	-0.663**	-0.192	-0.426	1	
Chloride	-0.530*	-0.111	0.306	0.493*	-0.019	0.146	-0.421	0.173	0.576**	1

Temp: water temperature; EC:electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K: potassium
***significant at the $p<0.05$**
****significant at the $p<0.01$**

Table 4: Pearson correlation between indicator and emerging pathogens

Winter season											
	THPC	TC	FC	*E.coli*	UTI bacteria	*V. cholera*	*S. enterica*	*S. flexneri*	*L. monocytogenes*	*A. Hydrophila*	*S. aureus*
THPC	1										
TC	0.504*	1									
FC	0.664**	0.833**	1								
E.coli	0.549**	0.563**	0.528*	1							
UTI bacteria	0.151	-0.790**	-0.740**	0.179	1						
V. cholera	0.468*	0.502*	0.654**	-0.292	0.059	1					
S. enteric	0.774**	-0.011	0.061	0.092	-0.046	0.481*	1				
S. flexneri	0.684**	0.077	0.233	0.363	-0.209	0.463*	0.463*	1			
L.monocytogenes	-0.031	0.028	-0.004	0.401	-0.121	-0.319	-0.298	0.377	1		
A. hydrophila	0.704**	0.059	-0.112	-0.495*	-0.059	0.761**	0.560**	0.436*	-0.334	1	
S. aureus	-0.303	0.812**	0.907**	-0.065	-0.820**	-0.025	0.093	0.038	-0.163	-0.166	1
Summer season											
THPC	1										
TC	0.584**	1									
FC	0.742**	0.898**	1								
E.coli	0.606**	0.543*	0.643**	1							
UTI bacteria	-0.577**	-0.777**	-0.756**	-0.109	1						
V. cholera	0.594**	0.568**	0.602**	-0.194	-0.235	1					
S. enteric	0.092	0.309	0.083	0.158	-0.239	0.280	1				
S. flexneri	-	-	-	-	-	-	-	1			
L.monocytogenes	-0.079	-0.659**	-0.445*	-0.042	0.263	-0.602**	-0.122	-	1		
A. hydrophila	0.258	0.097	0.051	-0.776**	-0.292	0.319	-0.261	-	-0.332	1	
S. aureus	0.069	0.481*	0.278	-0.380	-0.782**	-0.032	0.282	-	-0.276	0.332	1

THPC: Total Heterotrophic Plate Count; TC: Total Coliform; FC: Fecal Coliform; UTI bacteria: Urinary Tract Infection bacteria
***significant at the p<0.05**
****significant at the p<0.01**

Table 5: Pearson correlation between physicochemical parameters and microbial indicator/pathogens

Winter season										
	pH	**Temp.**	**EC**	**BOD**	**DO**	**Nitrate**	**Ca+Mg**	**Na^+**	**K^+**	**Chloride**
THPC	0.634**		-0.738**		-0.750**	-0.651**		0.436*		
TC	0.581**	-0.448*		0.556*	-0.567**	0.529*		0.576**		
FC	0.655**				-0.536**					
E.coli	0.660**			0.785**			-0.608**	0.478*	0.453*	-0.692**
UTI bacteria	0.109*	0.543*						0.809**		
V. cholera	0.574**			0.608**			-0.744**			0.661**
S. enteric		-0.610*	-0.596**	0657**	-0.718**	0.730**				
S. flexneri	0.594**									
L.monocytogenes									-0.195**	-0.602**
A. hydrophila			0.586**	0.517*	-0.441*		-0.640**		0.452*	0.525*
S. aureus		-0.454*	-0.458*					-0.558**		
Summer season										
THPC	0.811**		-0.594**	0.484*	-0.525*	-0.536*				
TC	0.531*	-0.575*		0.514*	-0.587**	0.509*		-0.459*		
FC	0.779**				-0.560**					0.491*
E.coli	0.655**			0.780**			-0.748**	0.497*		-0.759**
UTI bacteria	0.720**	0.512*						0.560**		
V. cholera	0.514*			0.576**	-0.421*	0.552**				
S. enteric		-0.693**	-0.515*	0.680**	-0.411*	-0.535*				
S. flexneri										
L.monocytogenes					-0.160*	-0.546*		0.455*		-0.179*
A. hydrophila			0.621**	0.584**			-0.817**			0.610**
S. aureus		-0.454*						-0.523**		

Temp: water temperature; EC: electrical conductivity; BOD: biological oxygen demand;DO: dissolved oxygen; Ca: calcium; Mg:magnesium; Na: sodium; K: potassium; THPC: Total Heterotrophic Plate Count; TC: Total Coliform; FC: Fecal Coliform; UTI bacteria: Urinary Tract Infection bacteria
***significant at the $p<0.05$**
****significant at the $p<0.01$**

Table 6: Water quality index (WQI) based on physicochemical and biological parameters of water samples.

		Percentage of water samples	
Levels	**Water quality index score**	**Winter**	**Summer**
Level 1	0-25: Extremely polluted	99%	100%
Level 2	26-50: Suitable for transportation purposes	1%	0
Level 3	51-75: Suitable for irrigation purposes	0	0
Level 4	76-90: Suitable for domestic usage	0	0
Level 5	91-100: Suitable for domestic water supply	0	0

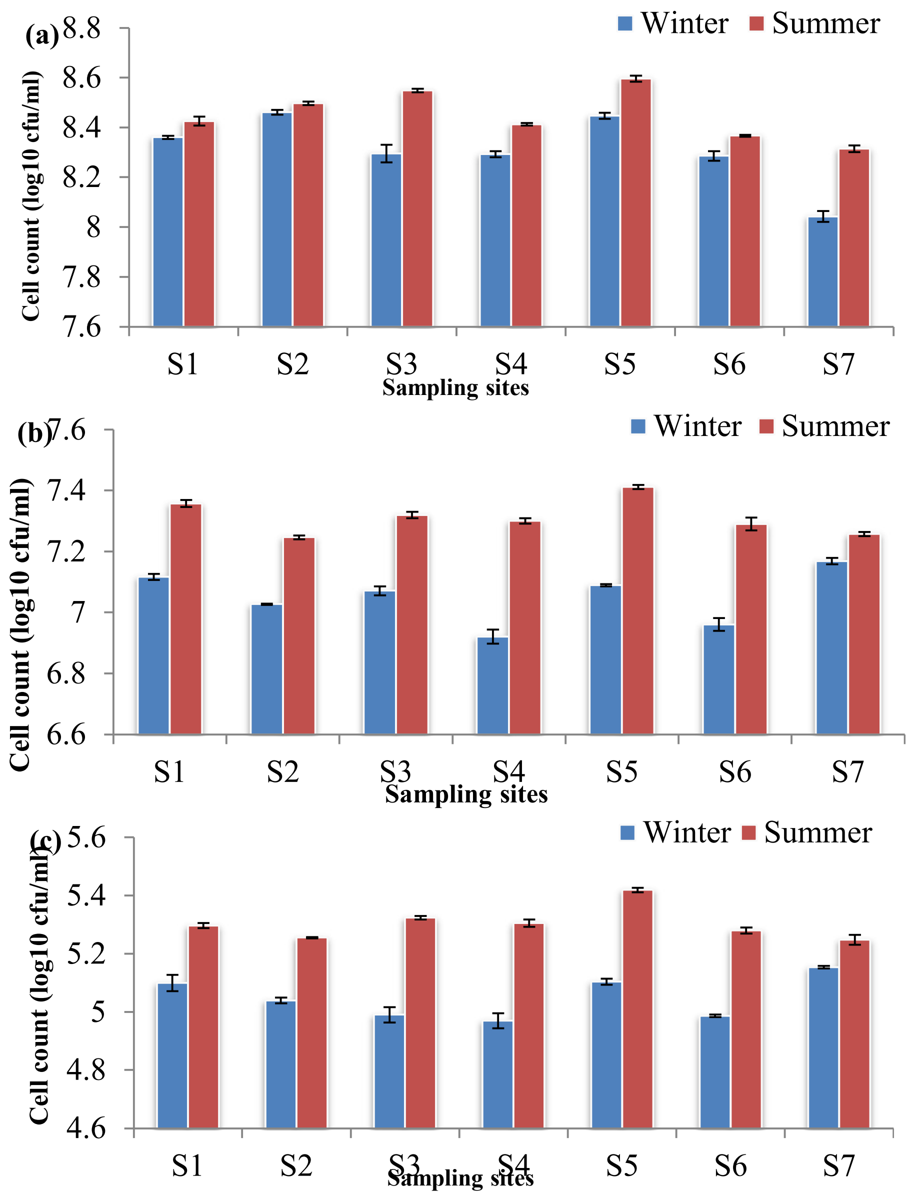

Figure 2: Bacterial cell count (log10 cfu/ml) of (a) Total Heterotrophic plate count (THPC); (b) Total Coliform (TC) and (c) Fecal Coliform in winter and summer season

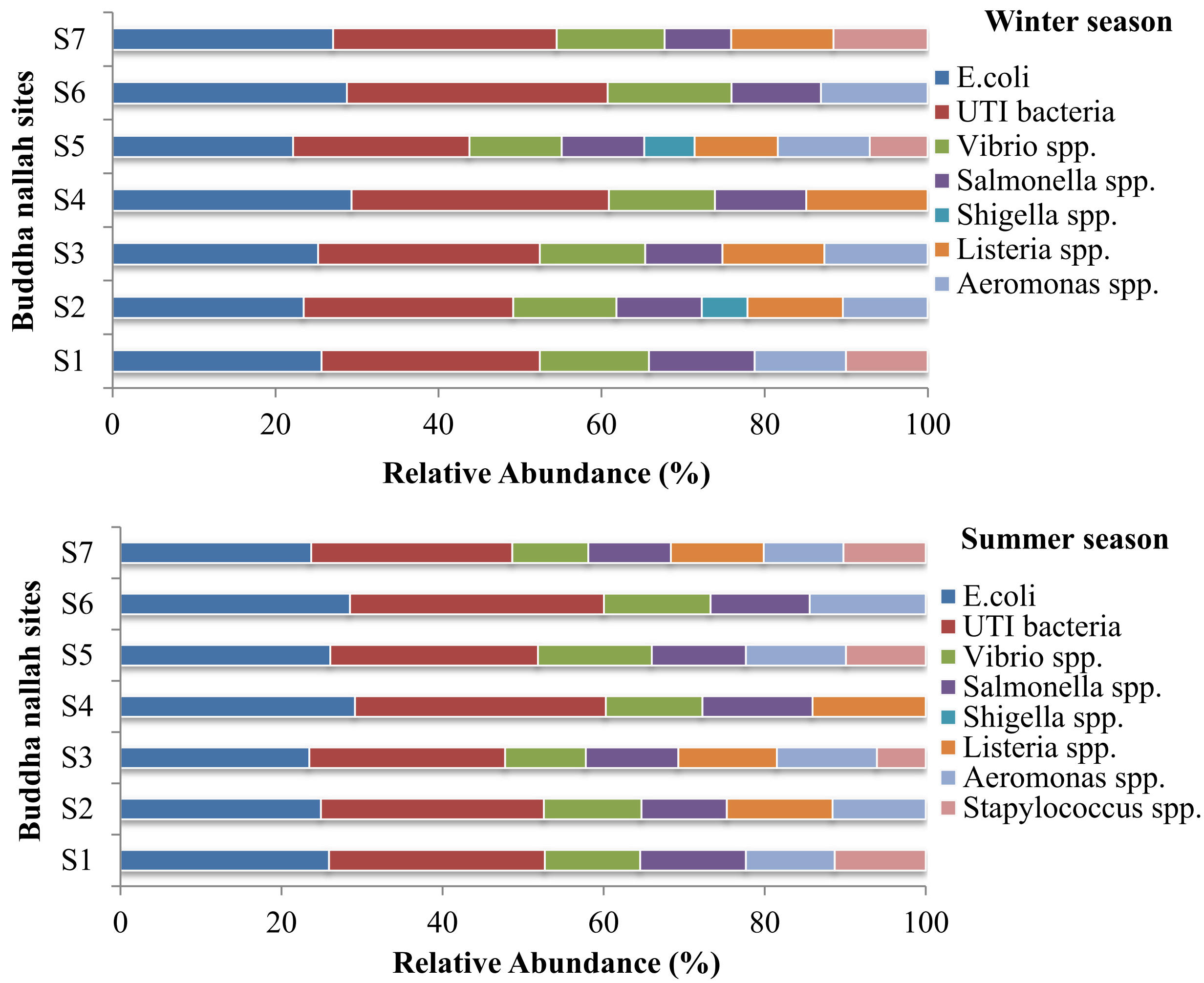

Figure 3: Relative abundance of water pathogens at different sites of Buddha nallah, Ludhiana, Punjab.

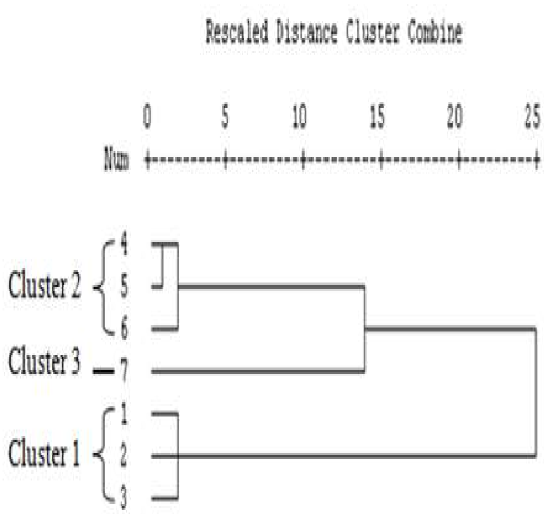

Figure 4:Clustral analyses of Buddha nallah sites (S1-S2) for winter season.

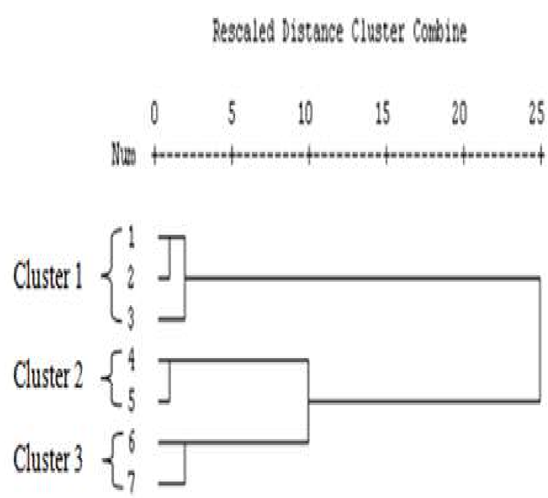

Figure 5: Clustral analyses of Buddha nallah sites (S1-S2) for summer season.

significant correlation was found between the Total Coliform (TC), Fecal Coliform (FC) and *E.coli.* In this study, the correlation between indicator organisms and emerging pathogens were found very weak and doesn't support the concept of using coliforms as a signal of presence of pathogens in the water quality assessment practices. Among all the pathogens, *Vibrio cholera* and *Staphylococcus aureus* were only emerging pathogens that were positively correlated with coliform (r =0.502-0.901).The results from this study revealed the overall weak correlations among the pathogens. In terms of the physicochemical parameters, an increase in pH and biological oxygen demand (BOD) significantly increased the prevalence of indicator/pathogens in water. While temperature, electrical conductivity (EC), dissolved oxygen (DO), nitrates, chlorides and nutrients showed the opposite trend. The correlation analysis also showed that with the seasonal variation there is no significant relation between the physicochemical parameters and microbial indicators/pathogens.

Clustral analysis

Clustral analysis (CA) is a multivariate statistical tool to classify different places or objects on the basis of distance and proximity. They classified different clusters on the basis of similarities and differences in their components. The dendrogram generated by the group linkages cluster analysis divided 7 Buddha Nallah sites into three cluster groups with significant differences between the groups. In summer as shown in Fig. 5, cluster 1 included sites S1 to S3; cluster 2 included S4-S6 sites and cluster 3 comprised only S7 site. Whereas in winter, cluster 2 comprised only two sites S4 and S5; cluster 3 includes S6 with S7 site as shown in Fig. 4. Therefore, the spatial and temporal change in Buddha Nallah water quality depends largely on local climate conditions (winter and summer) and pollutant sources (domestic, industrial and agricultural runoffs). Based on clustral analyses, the cluster 1 group included the sites that present the highly polluted areas, the cluster 2 group sites are moderately polluted areas and cluster 3 group sites in less polluted areas. In cluster 1, the main source of pollutants was discharges from textile, dairy and domestic wastes. The cluster 2 sites were mainly polluted with paper industry discharges. However due to strict actions for industrial discharges in Ludhiana, the wastewater discharged from paper mills should be reduced and cause moderate pollution. As compared to other areas, the cluster 3 is somehow less populated as it was away from industrialized and urbanized areas.

Water Quality Index (WQI)

Results in Table 6 represent the water quality index based on physicochemical and biological parameters of water samples during the winter and summer season. The classification of water quality into different levels was adapted from VEA 2011. Based on the WQI assessment, 99% and 100% of the Buddha Nallah water samples was found to be extremely polluted during winter and summer season, respectively. These results revealed that the water of Buddha Nallah is not suitable for domestic or irrigation purposes because it poses high public health risks.

Conclusion

High electrical conductivity (EC) in upstream as compared to the downstream sites clearly indicate that water contain high amount of dissolved solids and is highly polluted with industrial and domestic discharges. There was no significant difference in the pathogen abundance for the two seasons except for the *S. flexneri* which had significantly higher abundance in winter season. The correlation analysis showed that with the seasonal variation there is no significant relation between the physicochemical parameters and microbial indicators/pathogens. Based on clustral analyses, the cluster 1 group included the sites that present the highly polluted areas, the cluster 2 group sites are moderately polluted areas and cluster 3 group sites in less polluted areas. Only 1% of water samples during the winter were categorized under level 2. The quality of water evaluated from WQI score indicates that water samples contain highlevel contamination and suggests the prevalence of water-borne pathogens in Buddha Nallah.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Anonymous.(2014).*"India's Manchester"*. *BBC*. 28 February 2006. Archived from the original on 18 August 2014. Retrieved 25 May 2014.

APHA &AWWA, W. P. C. F. (1998). Standard methods for the examination of water and wastewater 20th edition. *American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC*.

APHA AWWA,W.E.F. (2012). Standard Methods for the Examination of Water and Wastewater, 22nd edition.*American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC*.

APHA, &AWWA. (1971). *Standard methods for the examination of water and wastewater*. American Public Health Association.

Bhatia, D., Sharma, N. R., Kanwar, R., & Singh, J. (2018). Physicochemical assessment of industrial textile effluents of Punjab (India). *Applied Water Science*, *8*(3), 1-12.

Bhutiani, R., Ahamad, F., & Ram, K. (2021a). Quality assessment of groundwater at laksar block, haridwar in uttarakhand, India using water quality index: a case study. *Journal of Applied and Natural Science*, *13*(1), 197-203.

Bhutiani, R., Ahamad, F., & Ruhela, M. (2021b). Effect of composition and depth of filter-bed on the efficiency of Sand-intermittent-filter treating the Industrial wastewater at Haridwar, India. *Journal of Applied and Natural Science*, *13*(1), 88-94.

Bhutiani, R., Ahamad, F., Tyagi, V., & Ram, K. (2018). Evaluation of water quality of River Malin using water quality index (WQI) at Najibabad, Bijnor (UP) India. *Environment Conservation Journal*, *19*(1&2), 191-201.

BIS. (2012). Indian standard drinking water specification. *Bureau of Indian Standards*. 10500.

Das, S., Kaur, S., & Jutla, A. (2021). Earth Observations Based Assessment of Impact of COVID-19 Lockdown on Surface Water Quality of Buddha Nala, Punjab, India. *Water*, *13*(10), 1363.

Goh, S. G., Saeidi, N., Gu, X., Vergara, G. G. R., Liang, L., Fang, H., & Gin, K. Y. H. (2019). Occurrence of microbial indicators, pathogenic bacteria and viruses in tropical surface waters subject to contrasting land use. *Water research*, *150*, 200-215.

Jindal, R., & Sharma, C. (2011). Studies on water quality of Sutlej River around Ludhiana with reference to physicochemical parameters. *Environmental monitoring and assessment*, *174*(1), 417-425.

Kaur, J., Kaur, V., Pakade, Y. B., & Katnoria, J. K. (2021). A study on water quality monitoring of Buddha Nullah, Ludhiana, Punjab (India). *Environmental Geochemistry and Health*, *43*(7), 2699-2722.

Koneman, E., Allen, S., Janda, W., Schreckenberger, P., & Winn, W. (1983). *Diagnóstico microbiológico* (No. 950-06-1250-X. 04-A2 LU. CG-52.).

Koster, W., Egli, T., Ashbolt, N., Botzenhart, K., Burlion, N., Endo, T., Grimont, P., Guillot, E., Mabilat, C., Newport, L., & Niemi, M. (2003). Analytical methods for microbiological water quality testing. *Assessing microbial safety of drinking water*, p.237.

Matta, G., Srivastava, S., Pandey, R. R., & Saini, K. K. (2017). Assessment of physicochemical characteristics of Ganga Canal water quality in Uttarakhand. *Environment, development and sustainability*, *19*(2), 419-431.

Mavukkandy, M. O., Karmakar, S., & Harikumar, P. S. (2014). Assessment and rationalization of water quality monitoring network: a multivariate statistical approach to the Kabbini River (India). *Environmental Science and Pollution Research*, *21*(17), 10045-10066.

Mena-Rivera, L., Salgado-Silva, V., Benavides-Benavides, C., Coto-Campos, J. M., & Swinscoe, T. H. (2017). Spatial and seasonal surface water quality assessment in a tropical urban catchment: Burío River, Costa Rica. *Water*, *9*(8), 558.

Prasad, V. R., Srinivas, T. N. R., & Sarma, V. V. S. S. (2015). Influence of river discharge on abundance and dissemination of heterotrophic, indicator and pathogenic bacteria along the east coast of India. *Marine pollution bulletin*, *95*(1), 115-125.

Ruhela, M., Sharma, K., Bhutiani, R., Chandniha, S. K., Kumar, V., Tyagi, K., ... & Tyagi, I. (2022). GIS-based impact assessment and spatial distribution of air and water pollutants in mining area. *Environmental Science and Pollution Research*, *29*(21), 31486-31500.

Ruhela, M., Singh, V. K., & Ahamad, F. (2021). Assessment of groundwater quality of two selected villages of Nawada district of Bihar using water quality index. *Environment Conservation Journal*, *22*(3), 387-394.

Sharma, R., Kumar, R., Satapathy, S. C., Al-Ansari, N., Singh, K. K., Mahapatra, R. P., ... & Pham, B. T. (2020). Analysis of Water Pollution Using Different Physicochemical Parameters: A Study of Yamuna River. *Frontiers in Environmental Science*, *8*.

Sinha, S. N., Paul, D., & Biswas, K. (2016). Effects of Moringa oleifera Lam. and Azadirachta indica A. Juss. leaf extract in treatment of tannery effluent. *Our Nature*, *14*(1), 47-53.

USEPA. (2012). *Recreational Water Criteria*. Washington, DC: US Environmental Protection Agency Office of Water. 820-F-12-058.

VEA. (2011). Decision No. 879/ QĐ-TCMT Date 1/7/2011, *Guideline on the Calculation of Water Quality Index (WQI)*. Vietnam Environmental Administration, Hanoi.

WHO. (2008). Guidelines for drinking-water quality. *World Health Organization*.

WHO. (2011). Guidelines for drinking-water quality. *World Health Organization*, *216*, 303-304.

Winkler, L. W. (1888). The determination of dissolved oxygen in water. *Berlin DeutChem Gas*, *21*, 2843-2855.

Yisa, J., & Tijani, J.O. (2010). Analytical studies on water quality index of river Landzu.

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