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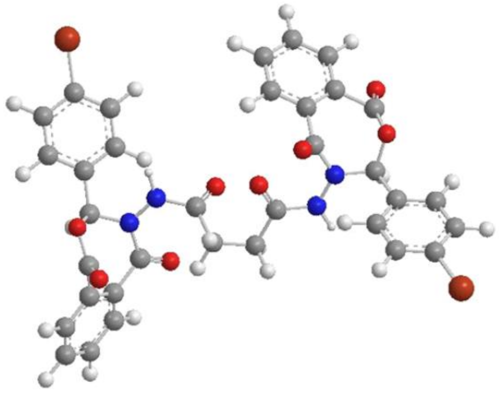

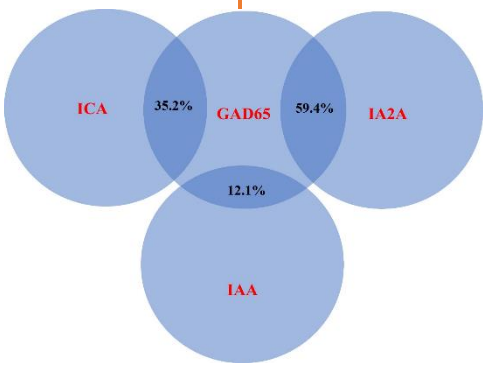

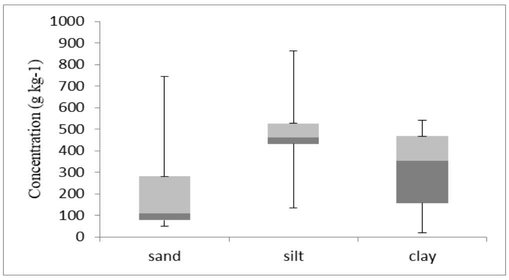

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Assessment of spatiotemporal variability of bacterial pollution in Darbandikhan Lake and its tributaries in the Kurdistan Region of Iraq

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Abstract

This study was designed to assess the spatiotemporal variability of bacterial pollution in Darbandikhan Lake and its river resources in Iraq's Kurdistan Region. Seasonally, 25 samples (a total of 100 samples year-round) were collected and examined for aerobic plate count (APC), total coliform (TC) and fecal coliform (FC) in all samples. Descriptive statistics for physiochemical and microbiological variables and the Pearson correlation coefficient (r) were performed using the Statistical Package for the Social Sciences (SPSS) version 20 and OriginPro 2022b–Correlation Plot. GraphPad Prism v9 was used to determine statistical significance ($p < 0.05$), one-way ANOVA was used, followed by Tukey's multiple comparison tests. Results indicated positive correlations between microbiological and Al, Cu, EC, Fe, K, Mg, Na, $NO3$, $PO4$, $SO4$, T.Alk, TDS, TH, Turb and Zn in most of seasons at the < 0.01 and < 0.05 significance levels. Furthermore, microbiological characteristics had a strongly positive correlation in all seasons. Water resources are heavily contaminated with coliforms, especially in wet seasons. The inhabitant's activities, untreated sewage and waste water, disposed waste, and livestock were the main contributors to this pollution, which in turn led to waterborne diseases, particularly diarrhea. Considering the above issues, the government should treat the water resources properly and take the required actions to solve this issue.

Introduction

Access to pure water has been recognized as a fundamental human right. Pure water is used for a range of purposes, including drinking, agriculture, industry, and the household, making its availability a crucial public health concern in modern society [1-3]. Microbial pollution in aquatic environments is a critical issue regarding the hygienic quality of water bodies. Bacterial pathogens are the leading cause of waterborne infections worldwide, particularly in many low-income nations, generating severe clinical disorders [4-7]. Much of the health problems in underdeveloped nations are caused by a lack of clean drinking water for over 2.6 billion people, which is responsible for around 2.2 million fatalities each year, 1.4 million of which are in children. This makes polluted potable water responsible for 80% of all diseases and more than one-third of all deaths in impoverished nations [8]. There are over 600 million cases of diarrhea and dysentery each year, and 46,000 newborns die due to dirty water and poor sanitation annually [1]. While the industrialized world has mostly solved this issue, access to pure water and sanitation is not the norm in the majority of resource-limited nations. It is anticipated that by 2050, almost six billion people will lack access to safe drinking water [2 & 9].

A variety of harmful microbes, including coliform groups (total and fecal coliforms and E. coli), can spread through drinking water and cause sickness in the population. Potential sources of fecal bacteria are classified into three categories: humans, livestock, and wildlife. A fourth category of pets or dogs may be introduced in more urban watersheds [10]. Because the intestinal habitats and selective pressures to which the bacteria are subjected vary from source to source, each source creates distinct types of fecal bacteria. Sewage treatment plant discharges and overflow from informal settlements are major variables influencing the microbiological quality of surface waterways [11]. Surface waterways running through cities are more polluted with fecal than their upstream catchments, and will increase particularly after rain events [12].

Coliform bacteria are the most significant indicators for assessing, monitoring, and predicting the microbiological quality of surface waterways [11 & 13-14]. Coliforms are enormous quantities of facultative anaerobiotic, gram-negative, lactose-fermenting, sporeless-producing rods found in the environment as well as in human and animal intestines. The coliform bacteria category comprises both infective and non-infective types and are widely employed as an indication of microbial contamination. Total Coliforms, which include bacteria found in soils, water, plants, and animals [15]. Coliforms including FC and E. coli, respond to the natural environment and treatment processes similarly to pathogens, which is why they are the most commonly used indicators of contamination because they are simple to recognize. A deeper examination of the coliform bacteria provides an evaluation of the quantity and concentration of infective bacteria in the sample [16-17].

Despite that, in the study area, Darbandikhan Lake and its water sources are mainly used for daily consumption for different purposes. Sewage wastewater and industrial discharge directly flow into rivers, and municipal solid waste disposal and commercial and industrial waste are spread throughout the area without treatment, and fertilizers and pesticides are used intensively. These sources could cause bacterial contamination and affect people around the study area and downstream of the dam.

Previous studies have demonstrated spatiotemporal variability of bacterial pollution and its consequences in different regions of the world [18-22]. The Tanjaro River, Darbandikhan Lake and downstream of the dam were evaluated in earlier studies [23-27], however, other tributaries and their impacts on the lake were mostly not assessed. This study aimed to determine the spatiotemporal variability of bacterial pollution (APC, TC, FC, and E. coli) in Darbandikhan Lake and its tributaries in Iraq's Kurdistan Region. It will identify the rivers pollution share ratio and sources of bacterial pollution.

Materials and methods

Study area

The Darbandikhan dam is resulted in the formation of the Darbandikhan Lake, is located 65 kilometers southeast of Sulaimani in the east of the Kurdistan Region - northeast of Iraq. It lays between latitude 35° 06′ 58″ and 35° 21′ 07″ N, and longitude 45° 40′ 59″ and 45° 44′ 42″ E (Figure: 1). The dam on the Sirwan River (Diyala River) was erected in 1956 and finished in 1961. The dam is 128 meters high, 445 meters long, and 17 meters wide at its widest point. The dam stands 532 meters above sea level (asl). The dam's storage capacity is 3 km^3, of which 2.5 km^3 is direct storage and 0.5 km^3 is dead storage, while the water is at its normal operating level in the dam (485m asl). At this level, the lake area is 113 km^2. This dam was built with a central mud core and a rock shoulder [28]. The Tanjaro, Chaqan, and Zalm Rivers flow from Iraq to the north and northwest of the lake, respectively, and the Sirwan and Zmkan Rivers flow from Iran to the east. The catchment area of the lake, including the lake, is 16788.74 km^2. The dam has two low-level outflow tunnels, 6 and 9 meters wide, that are used for irrigation. A spillway with three gates on the right bank with dimensions of 15 m by 15 m is also used for flood management. The spillway's maximum discharge capacity when working normally is 5,700 m^3/s. As the water level reaches to the design flood level (493.5 m asl), the spillway discharge is 11,400 m^3/s [29].

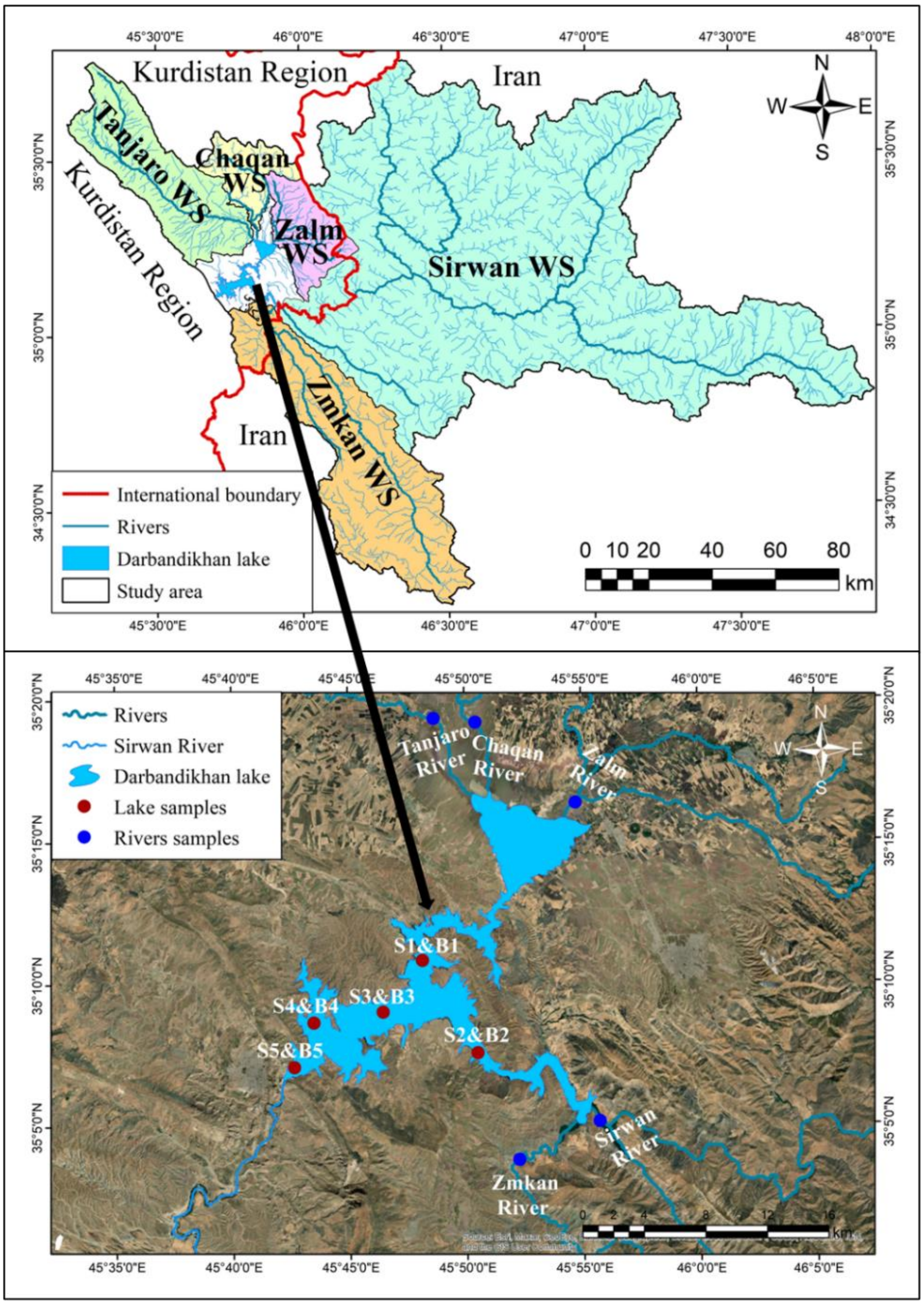

Figure 1: Locations of samples and study area [30]

Sample collection and analysis

From August 2021 to May 2022, twenty-five water samples were taken periodically. Fifteen samples were obtained downstream of the aforementioned rivers, and ten samples were collected from the lake (five samples from the subsurface and five samples from the bottom). The subsurface samples were obtained 1 m below the lake's surface, while the bottom samples were collected 1 m above the lake's surface (Table 1). The lake samples were collected using a Van Dorn bottle sampler. Table 1 displays the longitude and latitude of rivers and Darbandikhan Lake water sampling locations.

Table 1: Characteristics of sampling locations and their longitude and latitude

Type of samples	*Samples*	*X and Y*
Rivers	*Zmkan*	*45° 53' 02" E- 35° 04' 00" N*
	Sirwan	*46° 04' 45" E- 35° 05' 06" N*
	Zalm	*45° 53' 56" E- 35° 16' 21" N*
	Chaqan	*45° 51' 16" E- 35° 19' 11" N*
	Tanjaro	*45° 49' 03" E- 35° 19' 02" N*
Lake (Sub-surface and bottom)	*S1*	*45° 48' 09" E- 35° 10' 49" N*
	B1	
	S2	*45° 50' 30" E- 35° 07' 33" N*
	B2	
	S3	*45° 46' 27" E- 35° 09' 00" N*
	B3	
	S4	*45° 43' 29" E- 35° 08' 38" N*
	B4	
	S5	*45° 42' 38.5" E- 35° 07' 04" N*
	B5	

Water samples were collected from appointed locations, kept in 250ml Glass bottles in an ice box and analyzed in the laboratory. APC, TC and FC were determined in all samples. The rate of water contamination has been measured by the absence or presence of the above bacteria. APC was performed using a ten-fold serial dilution method and inoculated on nutrient agar and incubated at 37 °C for 24-48 hours ***[31]***. Total count is expressed as colony forming unit per 100 ml (cfu/mL). The most probable number (MPN) test is used to estimate the probable number of viable coliform groups present in the water samples. The principle of the MPN test depends on the lactose fermentation by the coliform group into acid and gas within Durham tubes. The decision of MPN is based on three successive steps, including:

a. The presumptive test involved mixing 10 ml of water sample in five tubes of double-strength MacConkey broth and the tubes were incubated for 24 hours at 37 °C. The presence of acid and gas in any tube indicates positive detection of total coliform [22].
b. The confirmation test was then carried out by transferring 0.1 ml from the first step's positive tube into a fresh single-strength MacConkey broth tube and incubating for 24 hours at 44°C [32].
c. In a complete test: the positive growth was recognized as a positive fecal coliform. The test was finalized by inoculating on Eosin Methylene Blue (EMB) agar. The appearance of a green metallic sheen on EMB agar indicates the presence of thermotolerant E. coli [33].

Furthermore, physiochemical parameters were calculated to assess the relationship between microbiological characteristics and physiochemical elements. The potential of hydrogen (pH), electrical conductivity (EC) and total dissolved hardness (TDS) were measured directly in the field by using Ph, EC and TDS meters. In the laboratory, EDTA-Titrimetry was used to determine calcium (Ca), magnesium (Mg), total hardness (TH, as $CaCO_3$) and total alkalinity (T.Alk, as $CaCO_3$). Turbidity was measured by a turbidity meter. Chloride (Cl) was determined by titrimetry. Nitrate (NO_3), phosphorus (PO_4) and sulphate (SO_4) were determined by spectrophotometry. Potassium (K) and sodium (Na) were determined by flame photometry. Aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), strontium (Sr), titanium (Ti), thallium (Tl), vanadium (V) and zinc (Zn) were measured after digestion by HNO_3 using a Multi Elements Standard QC 22E–NIST SRM (iCAP 7600 Dual/ICP-OES (Thermo Fisher). For these tests, 5 mL/L of HNO_3 were added to the water samples directly in the field.

Statistical analysis

The descriptive statistic was used to show the minimum, maximum, mean, and standard deviation physiochemical and bacterial parameters (*Table: 2*). The Pearson correlation was performed to test relationships among variables using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM Inc, USA) and OriginPro 2022b – Correlation Plot. GraphPad Prism v9 was used to determine statistical significance ($p < 0.05$), one-way ANOVA was used, followed by Tukey's multiple comparison tests.

River bacterial pollution share ratio

Depending on the APC results of collected samples from rivers, the river bacterial pollution share ratio in Darbandikhan Lake was estimated using the following equations[*]:

$$RBPSR = \frac{\mathrm{RAPCR}}{\Sigma\mathrm{RAPCR}} \times 100$$

$$RAPCR = \frac{\mathrm{RAPC}}{100} \times QR$$

$$QR = \frac{\mathrm{Q}}{\sum \mathrm{Q}} \times 100$$

Where RAPCR = river aerobic plate count ratio, RAPC = river aerobic plate count, QR = river discharge ratio and Q = river discharge.

Results and discussion

Statistical analysis

The Person's correlation coefficients (r) for the parameters were used to build the correlation matrix, which showed the relationship between physicochemical variables and microbiological characteristics, and seasonal variations of microbiological characteristics. The results of the selected parameters showed several substantial positive correlations at the < 0.01 and < 0.05 significance levels in all seasons (*Figure: 2*). Seasonal changes may be made on parameter levels and may vary from one season to another. In summer, APC had positive correlations at the < 0.01 significance levels with Al, Co, EC, Fe, NO3, PO4, Se, TDS and Turb and positive correlations at the < 0.05 significance levels with Mg, T.Alk, Temp, TH and Ti. In Autumn, APC had positive correlations at the < 0.01 significance levels with Ca, Cu, EC, K, Na, PO4, SO4, T.Alk, TH, TDS and Zn and positive correlations at the < 0.05 significance levels with NO3. In winter, APC had positive correlations at the < 0.01 significance levels with Cl, EC, K, Na, PO4, T.Alk and TDS and positive correlations at the < 0.05 significance levels with Mg, TH and Zn. In spring, APC had positive correlations at the < 0.01 significance levels with Al, Cr, Cu, EC, Fe, K, Na, Ni, NO3, Pb, PO4, SO4, T.Alk, TDS and Turb and positive correlations at the < 0.05 significance levels with Cd. Seasonal variations in parameter intensity caused these changes in parameter pair correlations.

Table 2: Descriptive statistics of physiochemical and APC

Seasons	*Summer*								*Autumn*							
Locations	***Rivers***				***Lake***				***Rivers***				***Lake***			
Parameters	***Min***	***Max***	***Ave***	***SD***	***Min***	***Max***	***Ave***	***SD***	***Min***	***Max***	***Ave***	***SD***	***Min***	***Max***	***Ave***	***SD***
Al^{3+} (mg/L)	0.0000	0.6450	0.2447	0.2687	0.0000	0.1040	0.0284	0.0432	0.3240	27.9530	6.4393	8.1042	0.1810	17.4200	2.5693	5.3200
As^{3-} (mg/L)	0.0000	0.0060	0.0010	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0016	0.0020	0.0020	0.0040	0.0027	0.0007
Ba^{2+} (mg/L)	0.0060	0.2390	0.0657	0.0886	0.0190	0.0430	0.0301	0.0090	0.0300	0.1320	0.0642	0.0358	0.0520	0.1060	0.0646	0.0154
Ca^{2+} (mg/L)	76.0	122.0	95.7	14.3	62.6	76.0	70.6	4.0	55.0	96.0	78.8	13.7	48.0	80.0	61.3	8.8
Cd^{2+} (mg/L)	0.0000	0.0010	0.0001	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0003	0.0007	0.0000	0.0020	0.0010	0.0007
Cl^{-} (mg/L)	38.0	96.0	70.6	18.2	48.0	113.0	89.1	19.6	39.0	125.0	73.1	27.1	54.0	75.0	61.9	6.6
Co^{2+} (mg/L)	0.0001	0.0020	0.0011	0.0005	0.0010	0.0010	0.0010	0.0000	0.0000	0.0080	0.0028	0.0027	0.0000	0.0110	0.0016	0.0033
Cr^{2+} (mg/L)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0030	0.0360	0.0153	0.0121	0.0040	0.0870	0.0155	0.0254
Cu^{2+} (mg/L)	0.0000	0.0550	0.0104	0.0203	0.0000	0.0000	0.0000	0.0000	0.0550	0.1450	0.0835	0.0262	0.0370	0.0800	0.0619	0.0138
EC (µs/cm)	529.0	1097.0	684.3	215.5	369.0	423.0	398.2	18.5	474.0	1112.0	666.5	235.0	382.0	591.0	488.0	58.7
Fe^{2+} (mg/L)	0.0000	0.4150	0.0923	0.1598	0.0000	0.1500	0.0224	0.0476	0.1890	5.7490	2.0315	2.0980	0.1400	13.7550	1.9194	4.2020
K^{+} (mg/L)	8.9	10.8	9.8	0.6	9.2	10.9	9.9	0.6	1.0	10.5	5.1	4.0	2.3	3.0	2.5	0.2
Mg^{2+} (mg/L)	9.7	80.7	40.7	18.5	4.9	41.9	19.3	11.6	9.7	29.8	19.2	6.1	9.7	36.5	22.9	7.1
Mn^{2+} (mg/L)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0580	1.2020	0.3352	0.4465	0.0440	0.5560	0.1576	0.1627
Mo^{3+} (mg/L)	0.0000	0.0230	0.0055	0.0088	0.0020	0.0030	0.0025	0.0005	0.0000	0.0080	0.0032	0.0029	0.0010	0.0040	0.0027	0.0009
Na^{+} (mg/L)	22.1	24.8	23.2	0.9	22.1	24.3	23.5	0.8	19.0	85.0	51.4	24.4	27.0	33.0	30.3	2.2
Ni^{2+} (mg/L)	0.0000	0.0050	0.0010	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0220	0.0063	0.0089	0.0000	0.0450	0.0066	0.0140
NO^{3-} (mg/L)	1.5	39.3	13.4	12.6	1.5	10.2	3.5	3.1	5.0	16.4	9.6	4.1	3.6	5.8	4.2	0.6
Pb^{2+} (mg/L)	0.0000	0.0040	0.0008	0.0014	0.0000	0.0010	0.0001	0.0003	0.0040	0.0110	0.0073	0.0021	0.0040	0.0120	0.0078	0.0025
pH	7.8	8.2	8.0	0.1	7.1	8.2	7.7	0.4	7.4	8.0	7.7	0.3	7.1	8.0	7.7	0.3
PO_4^{3-} (mg/L)	0.2	14.1	2.9	5.5	0.4	0.8	0.5	0.1	0.3	7.9	1.7	2.4	0.2	0.4	0.3	0.1
Sb^{3+} (mg/L)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Se^{2-} (mg/L)	0.0000	0.0040	0.0015	0.0015	0.0000	0.0010	0.0001	0.0003	0.0000	0.0040	0.0011	0.0013	0.0010	0.0030	0.0021	0.0009
SO_4^{2-} (mg/L)	71.0	99.0	83.9	7.7	76.0	96.0	85.8	7.0	29.6	223.0	88.8	48.9	51.0	98.0	68.7	14.5

Sr^{2+} (mg/L)	0.1880	1.1590	0.6675	0.3385	0.4710	0.6220	0.5317	0.0470	0.2950	1.0390	0.6235	0.2503	0.5610	1.0790	0.6900	0.1486
T. Alk (mg/L)	280.0	560.0	400.7	83.4	240.0	430.0	315.0	63.1	316.0	600.0	389.7	69.1	240.0	302.0	285.8	21.4
Temp. (°C)	23.2	31.0	28.1	2.8	21.7	34.8	27.1	5.4	14.5	24.4	18.2	2.6	17.6	24.4	20.3	2.7
T. H (mg/L)	280.0	530.0	406.7	72.6	200.0	350.0	256.0	47.4	220.0	348.0	275.9	34.4	235.0	280.0	247.6	13.7
TDS (mg/L)	264.0	548.0	341.3	107.2	186.0	214.0	200.0	9.8	220.0	527.0	312.2	112.5	179.0	279.0	229.7	28.0
Ti^{2+} (mg/L)	0.0040	0.0120	0.0069	0.0035	0.0020	0.0040	0.0030	0.0007	0.0030	0.0410	0.0123	0.0097	0.0050	0.0440	0.0102	0.0120
Tl^{+} (mg/L)	0.0020	0.0070	0.0050	0.0013	0.0040	0.0060	0.0048	0.0008	0.0000	0.0020	0.0009	0.0005	0.0000	0.0010	0.0007	0.0005
Turb (NTU)	0.2	6.7	1.6	2.5	0.3	1.2	0.8	0.3	1.5	466.0	114.5	152.0	0.0	97.0	14.8	32.6
V^{2+} (mg/L)	0.0000	0.0950	0.0681	0.0356	0.0640	0.0810	0.0748	0.0053	0.0240	0.1130	0.0654	0.0402	0.0000	0.1380	0.0535	0.0403
Zn^{2+} (mg/L)	0.0780	0.1950	0.1054	0.0328	0.0170	0.0570	0.0292	0.0113	0.0390	0.4600	0.1571	0.1117	0.0990	0.1740	0.1399	0.0265
APC (CFU/100 mL)	4.93×10^{3}	3.57×10^{6}	7.24×10^{5}	1.59×10^{6}	1.40×10^{2}	7.60×10^{3}	2.91×10^{3}	3.19×10^{3}	1.13×10^{4}	3.47×10^{6}	9.83×10^{5}	1.46×10^{6}	4.00×10^{1}	1.40×10^{4}	3.60×10^{3}	4.60×10^{3}

Seasons	**Winter**								**Spring**							
Locations	**Rivers**				**Lake**				**Rivers**				**Lake**			
Parameters	**Min**	**Max**	**Ave**	**SD**	**Min**	**Max**	**Ave**	**SD**	**Min**	**Max**	**Ave**	**SD**	**Min**	**Max**	**Ave**	**SD**
Al^{3+} (mg/L)	0.0040	5.2080	0.9348	1.7508	0.0000	0.4110	0.1190	0.1521	0.0060	1.2970	0.4104	0.4373	0.0000	0.0160	0.0018	0.0050
As^{3-} (mg/L)	0.0000	0.0020	0.0003	0.0006	0.0000	0.0000	0.0000	0.0000	0.0030	0.0090	0.0064	0.0015	0.0040	0.0060	0.0053	0.0007
Ba^{2+} (mg/L)	0.0240	0.1570	0.0792	0.0391	0.0720	0.1040	0.0842	0.0107	0.0410	0.1570	0.0917	0.0412	0.0040	0.0880	0.0688	0.0249
Ca^{2+} (mg/L)	9.0	75.0	51.3	20.5	18.0	72.0	53.3	18.5	36.8	96.0	65.3	19.8	52.0	83.2	64.7	11.1
Cd^{2+} (mg/L)	0.0000	0.0010	0.0002	0.0004	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0010	0.0007	0.0000	0.0010	0.0003	0.0005
Cl^{-} (mg/L)	6.0	21.0	10.8	4.6	7.0	13.0	11.1	1.9	9.0	19.0	13.7	3.6	9.0	22.0	13.8	3.5
Co^{2+} (mg/L)	0.0000	0.0080	0.0021	0.0031	0.0000	0.0020	0.0006	0.0008	0.0000	0.0030	0.0013	0.0009	0.0000	0.0010	0.0004	0.0005
Cr^{2+} (mg/L)	0.0000	0.0770	0.0170	0.0285	0.0030	0.0120	0.0054	0.0030	0.0000	0.0050	0.0009	0.0019	0.0000	0.0000	0.0000	0.0000
Cu^{2+} (mg/L)	0.0000	0.0170	0.0045	0.0057	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0017	0.0020	0.0000	0.0010	0.0001	0.0003
EC (µs/cm)	400.0	1376.0	681.4	350.8	457.0	500.0	479.5	15.0	410.0	1065.0	669.8	235.4	395.0	451.0	414.8	15.5
Fe^{2+} (mg/L)	0.0190	12.1870	2.4322	4.5293	0.0000	1.3250	0.3567	0.4643	0.0100	1.6070	0.4916	0.5788	0.0000	0.0270	0.0055	0.0098
K^{+} (mg/L)	0.0	7.5	1.6	2.8	0.0	1.4	0.2	0.4	0.6	10.0	4.3	3.8	1.5	1.9	1.7	0.1
Mg^{2+} (mg/L)	24.0	80.0	51.2	19.8	33.0	60.0	49.4	8.5	33.7	75.1	51.6	12.5	46.4	73.0	56.0	8.4

Mn^{2+} (mg/L)	0.0000	0.3590	0.1361	0.1423	0.0000	0.3180	0.0760	0.1223	0.0000	0.0910	0.0256	0.0344	0.0000	0.0330	0.0064	0.0128
Mo^{3+} (mg/L)	0.0000	0.0030	0.0010	0.0009	0.0000	0.0030	0.0019	0.0009	0.0000	0.0090	0.0023	0.0034	0.0000	0.0020	0.0016	0.0007
Na^{+} (mg/L)	3.9	79.0	31.0	26.6	11.6	15.1	13.5	1.2	9.8	90.8	47.5	34.0	15.0	22.0	19.2	2.4
Ni^{2+} (mg/L)	0.0000	0.0540	0.0071	0.0184	0.0000	0.0000	0.0000	0.0000	0.0000	0.0120	0.0052	0.0053	0.0000	0.0010	0.0003	0.0005
NO^{3-} (mg/L)	1.0	9.0	2.6	2.2	1.0	6.0	1.9	1.5	3.4	30.1	16.2	9.2	4.0	8.1	5.9	1.6
Pb^{2+} (mg/L)	0.0020	0.0160	0.0073	0.0046	0.0030	0.0100	0.0062	0.0023	0.0030	0.0090	0.0047	0.0022	0.0010	0.0040	0.0026	0.0011
pH	7.6	8.8	8.1	0.3	7.0	8.2	7.7	0.3	7.2	8.7	7.8	0.5	7.3	8.4	7.9	0.5
PO_4^{3-} (mg/L)	0.3	5.1	1.6	1.7	0.3	0.6	0.4	0.1	0.2	9.7	2.8	3.6	0.2	0.8	0.4	0.2
Sb^{3+} (mg/L)	0.0000	0.0040	0.0008	0.0013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0001	0.0003	0.0000	0.0000	0.0000	0.0000
Se^{2-} (mg/L)	0.0000	0.0070	0.0027	0.0029	0.0000	0.0030	0.0007	0.0009	0.0000	0.0030	0.0008	0.0012	0.0000	0.0030	0.0011	0.0010
SO_4^{2-} (mg/L)	22.0	120.0	60.3	38.3	26.0	71.0	60.1	13.6	28.1	92.2	57.1	19.4	33.6	59.4	44.7	7.2
Sr^{2+} (mg/L)	0.2220	1.9120	0.7113	0.5382	0.5870	0.8060	0.7452	0.0751	0.3360	1.3300	0.7845	0.3038	0.0010	0.8060	0.6754	0.2415
T. Alk (mg/L)	190.0	480.0	262.9	103.7	138.0	220.0	182.2	22.7	206.0	428.0	282.1	80.4	134.0	220.0	172.6	33.3
Temp. (°C)	11.5	16.5	14.1	1.4	9.4	16.1	12.7	2.6	20.1	27.0	24.3	1.9	22.0	34.7	28.1	5.4
T. H (mg/L)	132.0	352.0	240.3	77.2	160.0	260.0	232.6	36.2	154.0	340.0	238.9	55.3	220.0	334.0	256.6	37.9
TDS (mg/L)	189.0	647.0	319.5	164.5	215.0	235.0	224.8	6.9	193.0	498.0	314.4	110.3	182.0	204.0	191.8	7.0
Ti^{2+} (mg/L)	0.0070	0.1930	0.0479	0.0745	0.0060	0.0230	0.0102	0.0056	0.0120	0.0180	0.0138	0.0019	0.0020	0.0140	0.0101	0.0031
Tl^{+} (mg/L)	0.0000	0.0040	0.0018	0.0014	0.0020	0.0050	0.0030	0.0009	0.0010	0.0040	0.0020	0.0008	0.0000	0.0030	0.0019	0.0010
Turb (NTU)	0.0	200.0	60.7	73.4	0.0	90.0	17.0	35.9	0.0	247.0	66.5	92.2	0.0	10.0	5.1	4.5
V^{2+} (mg/L)	0.0000	0.2150	0.0889	0.0711	0.0280	0.1040	0.0846	0.0264	0.0080	0.0380	0.0232	0.0093	0.0010	0.0300	0.0243	0.0083
Zn^{2+} (mg/L)	0.0350	0.1990	0.0901	0.0531	0.0270	0.1440	0.0841	0.0377	0.0180	0.2040	0.0686	0.0633	0.0010	0.0720	0.0415	0.0237
APC (CFU/100 mL)	2.73×10^{2}	8.67×10^{3}	2.08×10^{3}	3.68×10^{3}	4.00×10^{1}	6.00×10^{2}	2.52×10^{2}	1.85×10^{2}	5.10×10^{3}	5.13×10^{5}	1.12×10^{5}	2.24×10^{5}	3.20×10^{2}	6.60×10^{3}	2.09×10^{3}	1.99×10^{3}

Furthermore, microbiological characteristics in all seasons had a strongly positive (nearly perfectly positive) correlation (*Table: 3*). These highly significant positive correlations illustrate that the main sources for microbiological characteristics in the study area's surface water could be sewage and waste water, disposal waste and animal feces.

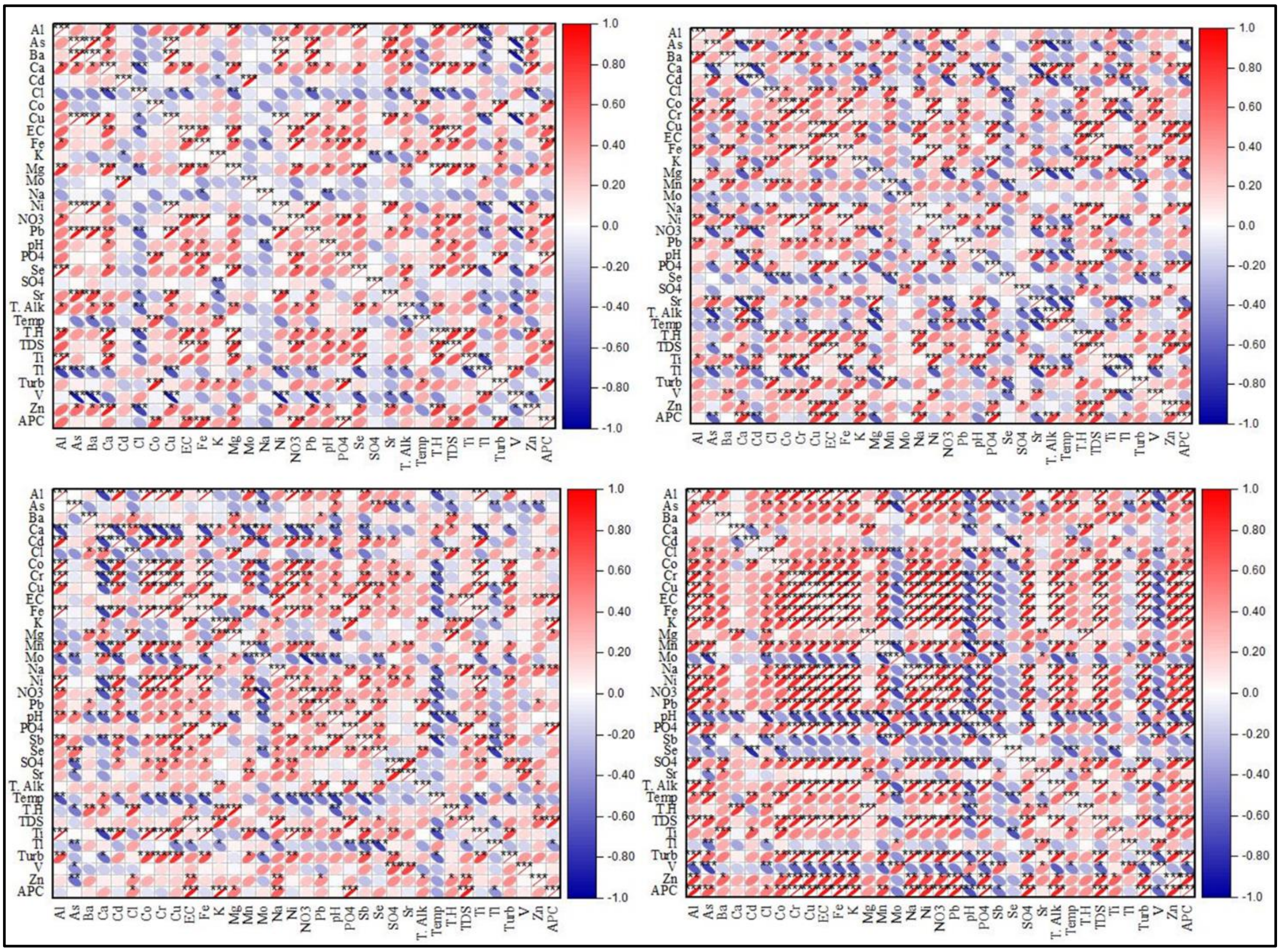

Figure 2: Correlation plot of the Pearson (*r*) values among variables for all seasons (summer top left, autumn top right, winter bottom left, spring bottom right). The *r* values from −1 to 1 are shown in blue to red (* p<=0.05 ** p<=0.01).

Table 3. Pearson Correlation coefficients (r) between microbiological characteristics for all seasons. ** Correlation is significant at the 0.01 level (2-tailed).

Seasons	*Summer*	*Autumn*	*Winter*	*Spring*
Summer	*1*	*0.951***	*0.996***	*0.999***
Autumn	*0.951***	*1*	*0.960***	*0.961***
Winter	*0.996***	*0.960***	*1*	*0.997***
Spring	*0.999***	*0.961***	*0.997***	*1*

Aerobic Plate Count (APC)

Aerobic plate count was used to reflecting overall drinking water quality and the existence of pathogens. APC is used as a bacterial population indicator in drinking water obtained from diverse sources. In these water samples, the microbial burden ranged from 4×10^1–3.57×10^6 CFU/100 mL (Table 2). The results revealed that bacterial contamination was observed in 100% of the water samples with spatiotemporal variability.

The number of CFUs in the Chaqan River water samples in all seasons was statistically significant at the level of P <0.0001 compared to other water samples collected in other rivers and the lake (Figure 3). There were no statistically significant differences in the number of CFUs in the water samples from the Zmkan, Sirwan, Zalm, and Tanjaro Rivers and the lake, which all had detectable levels of CFUs lower than 1.13×10^6.

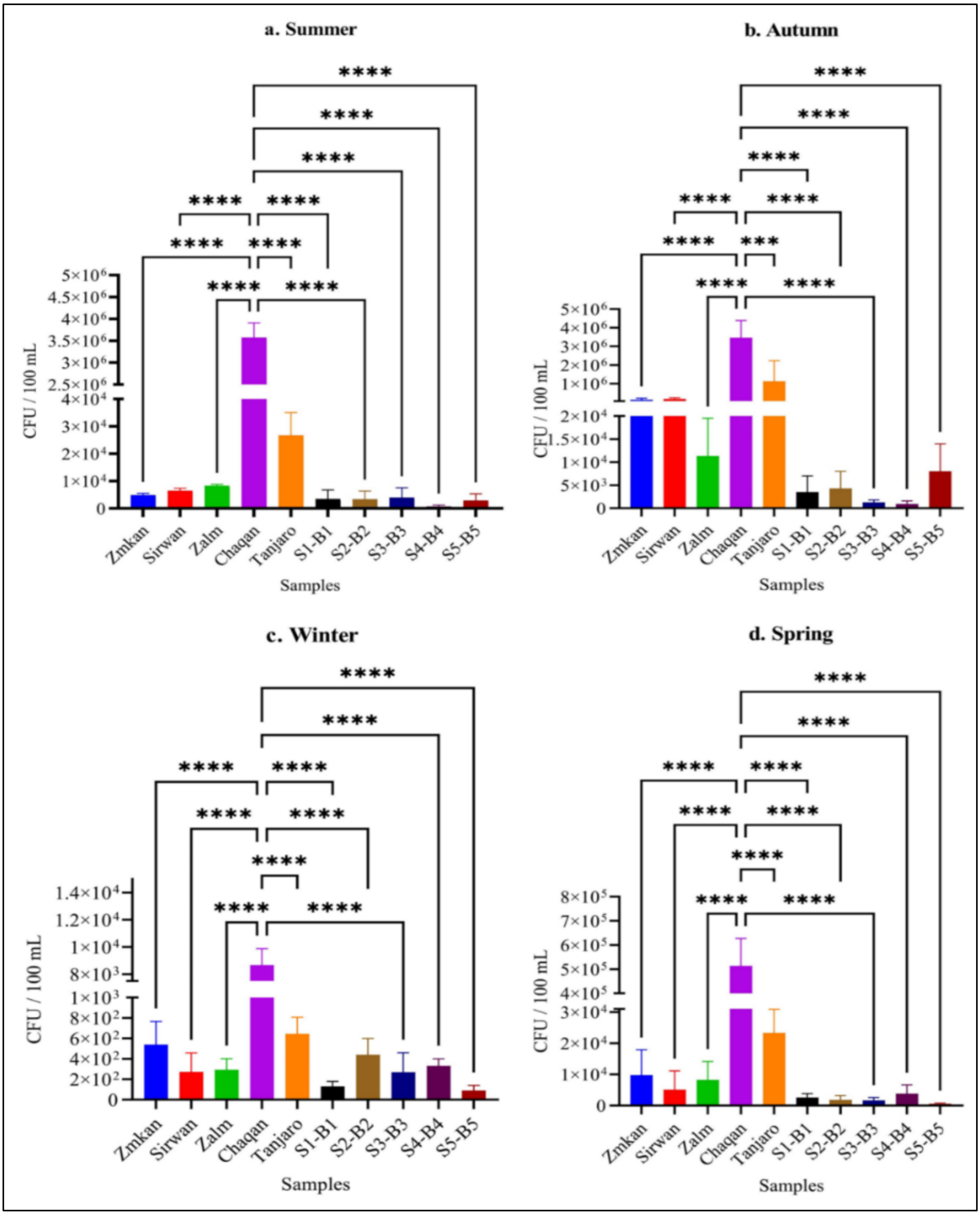

Figure 1: Seasonal APC variation in rivers and Darbandikhan Lake and using Tukey's multiple comparison tests (***P 0.0001 ****P <0.0001)

Total coliform (TC)

The results showed that all samples were contaminated by the TC bacterium at various levels (Table 4). All rivers' samples in all seasons had >16 MPN/100 mL. In the lake, samples S1, S2, B1, B2, B3, and B5 in the summer and autumn; S1, S2, B1, B2, and B3 in the winter; and S5 in the autumn had >16 MPN/100 mL, with the exception of S1 in the winter, which had 16 MPN/100 mL. S5 and B4 in summer, S4 and B4 in autumn, S5 in winter, and S1 in spring had 9.2 MPN/100 mL. All other samples in all seasons ranged from 2.2 MPN/100 mL to 5.1 MPN/100 mL. Overall, the spring had the lowest contamination, and the autumn had the highest contamination with TC.

Table 4. Seasonal variation in TC MPN index per 100 mL in the Zmkan, Sirwan, Zalm, Chaqan and Tanjaro rivers and Darbandikhan Lake

Seasons	***Samples***	***2.2***	***5.1***	***9.2***	***16***	***>16***
Summer	*Lake*	*1*	*1*	*2*	*0*	*6*
	Rivers	*0*	*0*	*0*	*0*	*15*
Autumn	*Lake*	*1*	*0*	*2*	*0*	*7*
	Rivers	*0*	*0*	*0*	*0*	*15*
Winter	*Lake*	*3*	*1*	*1*	*1*	*4*
	Rivers	*0*	*0*	*0*	*0*	*15*
Spring	*Lake*	*6*	*3*	*1*	*0*	*0*
	Rivers	*0*	*0*	*0*	*0*	*15*

Fecal coliform (FC)

The results revealed that 96% of the samples were contaminated by FC bacteria. FC was only absent in S3 and S4 in the winter and S4 and B3 in the spring (Figure 4).

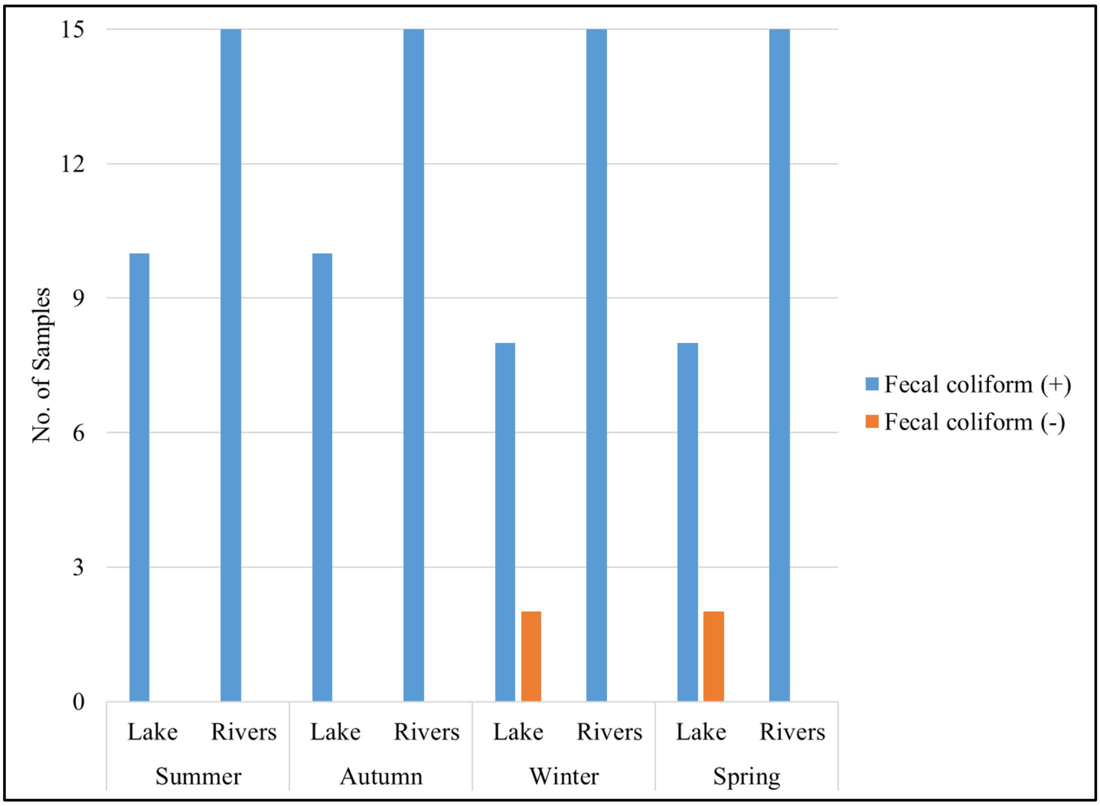

Figure 4: Seasonal variation in FC in the Zmkan, Sirwan, Zalm, Chaqan and Tanjaro rivers and Darbandikhan Lake

E. coli

E. coli was found in 68% of the water samples collected from the study area, and the other 32% were absent. 51 river samples (out of 60) tested positive, with the exception of Sirwan in the summer, Zalm in the autumn, and Tanjaro in the spring, which had negative results. 17 of the lake samples were positive, and 23 were negative. All lake samples were negative in the spring (Figure 5); however, all river and lake samples were positive in the winter with exception to S3 and S4 due to the rainfall pattern in the study area.

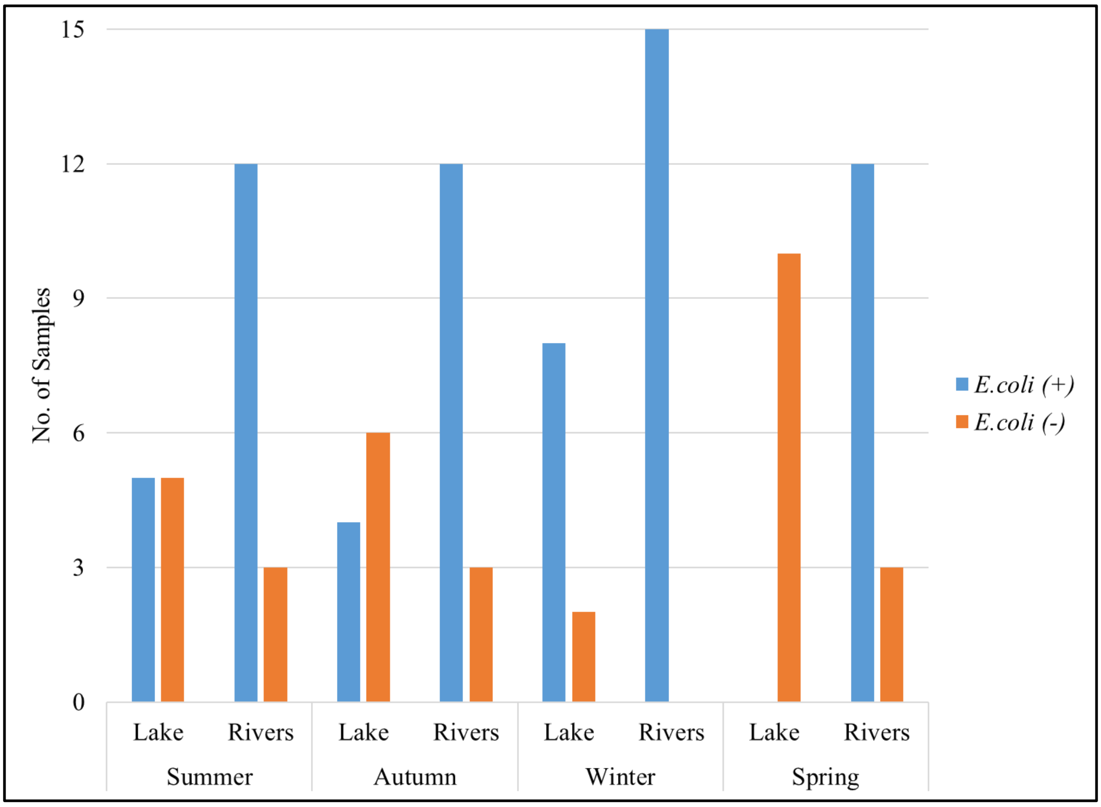

Figure 5: Seasonal variation in *E. coli* in the Zmkan, Sirwan, Zalm, Chaqan and Tanjaro rivers and Darbandikhan Lake

Coliform contamination of the water implies fecal contamination. The sources of coliforms in the study area surface water come from sewage, waste water and animal feces (*Figure: 6*). Sewage and waste waters are mixed with waterways directly without treatment due to the lack of treatment plants, making the waterways rich in coliforms (Table 5). Furthermore, domestic and wild animal feces could wash away with rainwater during the rainy season (mainly November to May) and mixed with surface water (Table 6). Animals are mainly raised by farmers in open areas and feed on natural grazing and waterways.

Table 5: Typical bacterial concentrations in raw and treated residential wastewater [34]

Bacterial group	***Raw sewage (number/liter)***	***Treated effluent (number/liter)***
TC	$10^7 - 10^9$	$10^6 - 10^8$
FC / E. coli	$10^6 - 10^8$	$10^5 - 10^7$

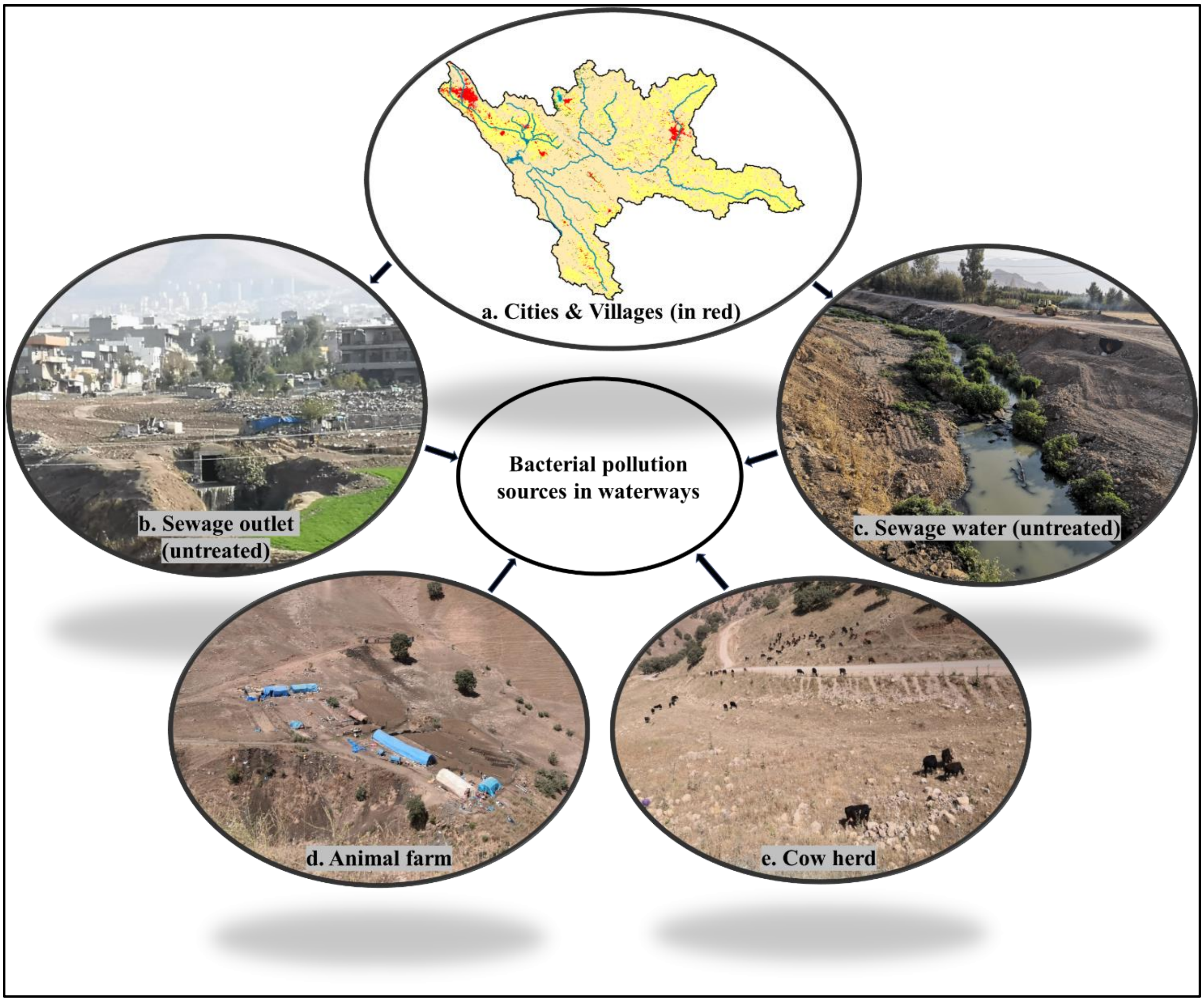

Figure 6: Simplified bacterial pollution sources in rivers and Darbandikhan Lake. (a) shows residential areas from cities and towns [35], and (b-e) shows some sources of bacterial contamination in the study area (photos were taken during field work).

Table 6: Quantity of *E. coli* bacteria found per gram feces of human and adult animals [36]

Human and animals	***Numbers/gram***
Cow	2×10^4
Horse	1.3×10^4
Pig	3.2×10^6
Sheep	3.2×10^6
Chicken	4×10^6
Dog	3.2×10^7
Cat	4×10^7
Human	5×10^6

The reduction in bacterial concentration in water resources will take time. In natural conditions, total coliforms require approximately one day to achieve a 50% reduction in concentration, whereas *E. coli* requires 1.5-3 days (Table 7).

Table 7: Reduction times for fecal bacteria in surface waters [34]

Bacteria group	*Time for half reduction in concentration (days)*
TC	*0.9*
E. coli	*1.5 – 3*

The findings of this study are mainly consistent with previous research carried out in the study area and other regions of the world. Some studies reported coliform contamination in the Tanjaro River [27] and Darbandikhan Lake [25]. Mohammed et al. (2020) investigated the prevalence of diarrhea in drinking water in Darbandikhan city. They concluded that tap water in Darbandikhan City that came from the Darbandikhan Lake had coliforms even after chlorination. They illustrated that 14 samples (out of 36 samples) had total coliform, seven samples had fecal coliform, and two samples had E. coli. Both above studies stated sewage, waste water and garbage as a contamination source. Also, downstream of the dam coliforms were recorded due to untreated waste water, discharge from sewage treatment plants and agricultural activities. Coliforms were recorded in the Diyala River during the winter [24], at the Al-Azizziyah site in the Tigris River [26] and Tigris River near the water purification stations within Baghdad Province [23]. Coliforms were recorded in the Southern Gulf of Lake Tana [22], in Mariout lake in Egypt [37], Ecuador's main rivers due to untreated sewage, animal wastes and population activities [38].

Previous research has conclusively shown that rainy seasons increase the number of coliforms in surface water. The highest value of E. coli (13.5 cfu/100 ml) was recorded in the eastern Himalayan state of Sikkim in the rainy season [20]. According to [21] 15 mm of rainfall in 24-h would increase E. coli three times compare before rainfall event and [2] mainly stated the same results.

Bacterial contamination in water can be harmful to human health. High TC and FC concentrations in water typically cause gastroenteritis, dysentery, diarrhea, viral hepatitis, and in rare cases, fever and other secondary problems. Bathing and swimming in surface waterways are very popular among local people [11 & 39], posing them at great risk of the above-mentioned infections.

E. coli is essential for digestion, nutritional absorption, and vitamin K and B synthesis. E. coli could thrive with or without oxygen because of its capacity to use carbohydrates as a source of energy. Despite being mutualistic with its host, many E. coli strains are harmful and can cause diseases such as urinary tract infections, sepsis or meningitis, and enteric or diarrheal disorders in immunocompromised individuals [17] and [40]. They may also cause nausea, headaches, and other side effects. Infants, young children, some of the elderly, and people with extremely weakened immune systems may be more vulnerable than the general population [1, 5, 15 & 41-43].

Regarding the study area [25] found that 161 diarrhea cases occurred in Darbandikhan city in July and August of 2019. fifty-three diarrheal cases (32.92%) among residents who used tap water (Darbandikhan Lake water after chlorination), and 47.83% of diarrheal conditions occurred among children. Similarly, [27] stated that the Tanjaro River pollution has an impact on people's quality of life by greatly increasing their likelihood of developing a variety of health issues, including kidney, respiratory, digestive, neurological, congenital, and intestinal issues, which are the most typical illnesses associated with such residents due to coliform bacteria. In other regions, [18] confirmed the connection between the prevalence of gastrointestinal disorders and the bacterial pollution of the Atoyac River. Typhoid, dysentery, vomiting, and other waterborne diseases are widespread in Bangladesh, and the emergence of antibiotic resistance among coliform bacteria through drinking water exacerbates the problem [19]. These problems would arise in the study area if the drinking water were contaminated with coliforms or if inhabitants and tourists drank directly from rivers such as the Sirwan and Zalm.

River bacterial pollution share ratio

The proportion of bacterial contamination in the rivers varied in both spatial and temporal dimensions. The Chaqan River, Tanjaro River, Sirwan River, Zmkan River and Zalm River took first to fifth place in bacterial pollution share ratio on an annual average. While the Sirwan River, Tanjaro River, Zmkan River, Zalm River and Chaqan River ranked from one to five in discharge amount, respectively. Tanjaro and Sirwan in the autumn had their highest pollution share ratios at 43.22% and 44.1%, respectively; Chaqan in the summer had the highest at 88.4%; and Zalm and Zmkan in the winter had their highest at 3.37% and 11.05%, respectively (*Figure: 7*). In contrast, Tanjaro, Sirwan, and Zmkan in the summer had their lowest pollution share ratios at 6.81%, 4.13%, and 0.39%, respectively, and Chaqan and Zalm had their lowest at 10.23% and 0.05%, respectively. These fluctuations in the bacterial pollution sharing ratio of rivers were mostly influenced by seasonal APC findings and discharge.

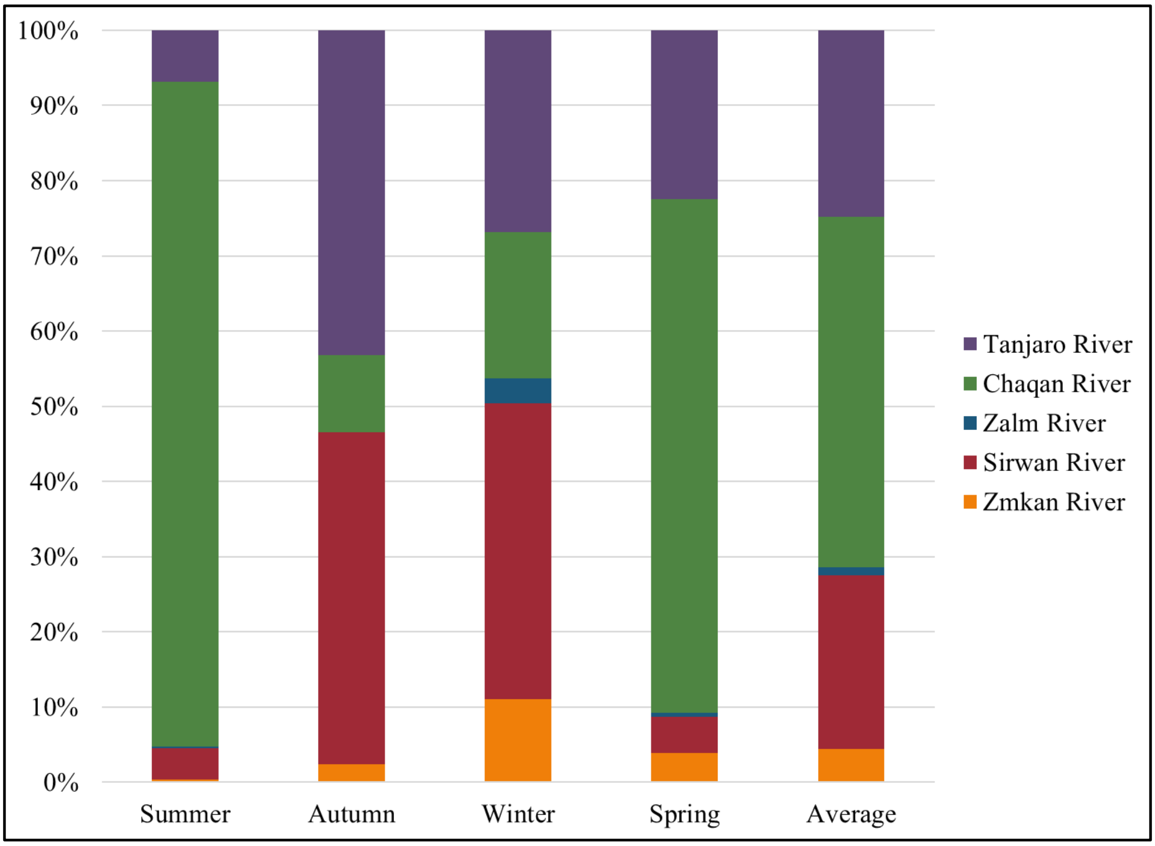

Figure 7: Seasonal and annual bacterial pollution share ratios of the Zmkan, Sirwan, Zalm, Chaqan, and Tanjaro rivers in the study area. Based on the seasonal river's APC results and discharge, the ratios were calculated.

Beside the river's discharge amount, the number of populations and their activities, the absence of water treatment facilities, the existence of landfills, and livestock farming are among the factors influencing this spatiotemporal heterogeneity in this bacterial pollution share ratios (Figure 6). In total, the study area has about 2.45 million inhabitants (Table: 8). The Sirwan River watershed has the highest population with 1134260 (46.41%) inhabitants, and the Zmkan watershed has the lowest population with 36332 (1.48%). This population engages in a wide range of activities, some of which directly affect the water quality of the rivers, including using river waters for daily consumption, mixing disposal waste, swage, and waste waters into rivers, and raising livestock around waterways.

Table 8: population distribution by watersheds in the study area [44] and [45]

Watershed	***Population***	***%***
Tanjaro River	*1049919*	*42.96*
Chaqan River	*107311*	*4.39*
Zalm River	*116215*	*4.76*
Sirwan River	*1134260*	*46.41*
Zmkan River	*36332*	*1.48*
Sum	*2444037*	*100*

It can be noted that the highest pollution share, like the highest APC values recorded in rainy seasons, with the exception of the Chaqan River, was recorded in the summer, when its discharge was 100% sewage water. This result is mainly consistent with [46], which stated that during high river discharge, higher microbial concentrations were found, whereas during low river discharge, both low and high microbial concentrations were found. Rainstorms increase water flows and river discharge, and these will lower the quality of the water. This may result in faster transmission of fecal pathogens from the source of contamination to abstraction sites. Under normal discharge conditions, water's self-purification happens through sedimentation, dilution, sunlight inactivation, predation, and starvation. Though self-purification becomes far less sufficient under high river discharge conditions [33].

Conclusions

Significant spatiotemporal variations in microbial pollution in Darbandikhan Lake and its tributaries were evident at different levels of concentration. The Chaqan River, Tanjaro River, Sirwan River, Zmkan River and Zalm River took first to fifth place in bacterial pollution share ratio on an annual average. The sources of this pollution could be linked to the population and their activities, untreated sewage and waste water, disposal waste, and livestock farming in the study area. The highest concentrations of bacterial pollution in the rivers were recorded in the rainy seasons. It is well known that these contaminating bacteria have the potential to cause a wide range of waterborne diseases. The findings reported here raise a great public health concern as the Darbandikhan Lake and some of its tributaries are drinking water sources for the surrounding cities. This necessitates immediate action by the government and health policymakers to control the contamination issue in these waters and further mitigate the risks.

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Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

References

1. Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., & Das, S. (2021). Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. Energy Nexus. 1 (100008), 1-9.
2. Abia, A. L. K., Ubomba-Jaswa, E., & Momba, M. N. B. (2015). Impact of seasonal variation on Escherichia coli concentrations in the riverbed sediments in the Apies River, South Africa. Science of the Total Environment. 537, 462-469.
3. Ashbolt, N. J. (2015). Microbial contamination of drinking water and human health from community water systems. Current environmental health reports. 2, 95-106.

4. Khan, S., Ali, S., Muhammad, S., Khan, B., Ali, A., Hesham, E. L., & Begum, S. (2020). Bacterial contamination in drinking water of urban Peshawar: a comparative study at the sources and user points of tube wells. Desalination and Water Treatment. 181, 221-227.

5. Gwimbi, P., George, M., & Ramphalile, M. (2019). Bacterial contamination of drinking water sources in rural villages of Mohale Basin, Lesotho: exposures through neighbourhood sanitation and hygiene practices. Environmental health and preventive medicine. 24 (33), 1-7.

6. Bylund, J., Toljander, J., Lysén, M., Rasti, N., Engqvist, J., & Simonsson, M. (2017). Measuring sporadic gastrointestinal illness associated with drinking water–an overview of methodologies. Journal of water and health. 15(3), 321-340.

7. Jung, A. V., Le Cann, P., Roig, B., Thomas, O., Baurès, E., & Thomas, M. F. (2014). Microbial contamination detection in water resources: interest of current optical methods, trends and needs in the context of climate change. International Journal of Environmental Research and Public Health. 11(4), 4292-4310.

8. Wilopo, W., & Putra, D. P. (2021). Groundwater recharge estimation using groundwater level fluctuation patterns in unconfined aquifer of Yogyakarta City, Indonesia. Kuwait Journal of Science. 48(2), 1-11.

9. US EPA. (2002). Wastewater Technology Fact Sheet, Bacterial Source Tracking. Office of Water, 832-F-02-010 200.

10. Antony, R. M., & Renuga, F. B. (2012). Microbiological analysis of drinking water quality of Ananthanar channel of Kanyakumari district, Tamil Nadu, India. Revista Ambiente & Água - An Interdisciplinary Journal of Applied Science. 7 (2), 42-48.

11. Reynolds, L. J., Martin, N. A., Sala-Comorera, L., Callanan, K., Doyle, P., O'Leary, C., Buggy, P., Nolan, T. M., O'Hare, G. M. P., O'Sullivan J. J., & Meijer, W. G. (2021). Identifying sources of faecal contamination in a small urban stream catchment: a multiparametric approach. Frontiers in Microbiology. 12, 1-12.

12. Manini, E., Baldrighi, E., Ricci, F., Grilli, F., Giovannelli, D., Intoccia, M., Casabianca, S., Capellacci, S., Marinchel, Nadia., Penna, P., Moro, F., Campanelli, Alessandra., Cordone, A., Correggia, M., Bastoni, D., Bolognini, L., Marini, M., & Penna, A. (2022). Assessment of spatio-temporal variability of faecal pollution along coastal waters during and after rainfall events. Water. 14(3), 1-16.

13. Lyons, B. P., Devlin, M. J., Hamid, S. A., Al-Otiabi, A. F., Al-Enezi, M., Massoud, M. S., Al-Zaidan, A.S., Smith, A.J., Morris, S., Bersuder, P. and Barber, J.L., Papachlimitzou, A. & Al-Sarawi, H. A. (2015). Microbial water quality and sedimentary faecal sterols as markers of sewage contamination in Kuwait. Marine pollution bulletin. 100(2), 689-698.

14. Raña, J. A., & Domingo, J. E. (2017). Contamination of coliform bacteria in water and fishery resources in manila bay aquaculture farms. The Philippine Journal of Fisheries. 24(2), 98-126.

15. Verhille, S. (2013). Understanding microbial indicators for drinking water assessment: interpretation of test results and public health significance. National collaborating centre for environmental health. Public Health Agency of Canada, 1-12.

16. World Health Organization (WHO). (2008). Guidelines for Drinking-water Quality. Incorporating 1st and 2nd Addenda, Volume 1, Recommendations, 3rd ed.; WHO: Geneva, Switzerland.

17. Rodríguez-Tapia, L., & Morales-Novelo, J. A. (2017). Bacterial pollution in river waters and gastrointestinal diseases. International journal of environmental research and public health. 14(479), 1-11.

18. Saha, T., Sultana, A., Mia, M. Y., Sarker, S., & Mahmud, S. (2019). Assessment of Microbial, Physicochemical Quality and Antibiotic Sensitivity Pattern of Bacteria of Drinking Water at Santosh, Tangail, Bangladesh. Advances in Microbiology. 9(12), 1023-1038.

19. Singh, A. K., Das, S., Singh, S., Pradhan, N., Gajamer, V. R., Kumar, S., Lepcha, Y.D. & Tiwari, H. K. (2019). Physicochemical parameters and alarming coliform count of the potable water of Eastern Himalayan state Sikkim: An indication of severe fecal contamination and immediate health risk. Frontiers in public health. 7, 1-17.

20. Tornevi, A., Bergstedt, O., & Forsberg, B. (2014). Precipitation effects on microbial pollution in a river: lag structures and seasonal effect modification. PloS one. 9(5), 1-10.

21. Sitotaw, B., Daniel, B., Kibret, M., & Worie, W. (2022). Seasonal Dynamics in Bacteriological and Physicochemical Water Quality of the Southern Gulf of Lake Tana. The Scientific World Journal. 2022, 1-18.
22. Al-Bayatti, K. K., Al-Arajy, K. H., & Al-Nuaemy, S. H. (2012). Bacteriological and physicochemical studies on Tigris River near the water purification stations within Baghdad Province. Journal of Environmental and Public Health. 2012, 1-8.
23. Hashim, A. G. (2018). Impact of Rustumiya Station for Sewage Water Treatment on Bacterial Pollution of Diyala River. Al-Kufa University Journal for Biology. Third International Scientific Conference, 1-6.
24. Mohammed, S. S., Hama-karim, Y. H., & Gubari, M. I. (2020). The prevalence of diarrhoea and drinking water quality in Darbandikhan city, Iraq. Tropical Journal of Natural Product Research. 4(8), 343-347.
25. Oleiwi, A. S., & Al-Dabbas, M. (2022). Assessment of contamination along the Tigris River from Tharthar-Tigris canal to Azizziyah, middle of Iraq. Water. 14(8), 1-20.
26. Rahman, A. K., Khazini, L., & Mohammad, A. Origins of Water Pollution in Tanjaro River. 3rd National Congress of Chemistry and Nanochemistry from Research to National Development, 1-6.
27. Azeez, D. R., Ahmad, F. M., & Karim, D. A. (2020). Monitoring of Water Level Fluctuations of Darbandikhan Lake Using Remote Sensing Techniques. Plant Archives. 20(2), 901-906.
28. World Bank. (2006). Dokan and Derbendikhan Dam Inspections. Project number: 5062018, Final Report for: World Bank Preparation, Review and Authorisation Revision.
29. Arcgis online base map World Imagery. (2022). Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.
30. Miles, A. A., Misra, S. S., & Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. Epidemiology & Infection. 38(6), 732-749.
31. Leong, S. S., Ismail, J., Denil, N. A., Sarbini, S. R., Wasli, W., & Debbie, A. (2018). Microbiological and physicochemical water quality assessments of river water in an industrial region of the northwest Coast of Borneo. Water. 10(11), 1-12.
32. Yasin, M., Ketema, T., & Bacha, K. (2015). Physico-chemical and bacteriological quality of drinking water of different sources, Jimma zone, Southwest Ethiopia. BMC research notes. 8, 1-13.
33. Medema, G. J., Shaw, S., Waite, M., Snozzi, M., Morreau, A., & Grabow, W. (2003). Catchment characterisation and source water quality. Assessing Microbial Safety of Drinking Water. Chapter 4, 111-158. IWA Publishing, Cornwall, UK.
34. Karra, K., Kontgis, C., Statman-Weil, Z., Mazzariello, J. C., Mathis, M., & Brumby, S. P. (2021, July). Global land use/land cover with Sentinel 2 and deep learning. In 2021 IEEE international geoscience and remote sensing symposium IGARSS (pp. 4704-4707). IEEE.
35. North Carolina National Estuarine Research Reserve (NCNERR). (2003). Addressing Microbial Pollution in Coastal Waters: A Reference for Local Governments. Produced for the North Carolina Coastal Nonpoint Source Program, public document.
36. Farouk, A. E., AA Abdel-Hamid, E., & MT, M. (2020). Environmental studies on water quality, plankton and bacterial community in Mariout lake, Egypt. Egyptian Journal of Aquatic Biology and Fisheries. 24(4), 139-158.
37. Vinueza, D., Ochoa-Herrera, V., Maurice, L., Tamayo, E., Mejía, L., Tejera, E., & Machado, A. (2021). Determining the microbial and chemical contamination in Ecuador’s main rivers. Scientific reports. 11(1), 17640.
38. Khan, S., Shahnaz, M., Jehan, N., Rehman, S., Shah, M. T., & Din, I. (2013). Drinking water quality and human health risk in Charsadda district, Pakistan. Journal of cleaner production. 60, 93-101.
39. Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. International journal of environmental research and public health. 7(10), 3657-3703.
40. Hasan, M. K., Shahriar, A., & Jim, K. U. (2019). Water pollution in Bangladesh and its impact on public health. Heliyon. 5(8), 1-23.
41. Skipton, S. O., Bruce I. Dvorak, W. E., Woldt & Sherry L. W. (2014). Drinking Water: Bacteria. University of Nebraska–Lincoln Extension, Institute of Agriculture and Natural Resources, G1826, 1-4.

42. Rock, C., & Rivera, B. (2014). Water quality, E. coli and your health. The University of Arizona-College of Agriculture and Life Sciences. Cooperative Extension, 1-4.
43. Kurdistan Regional Government. (2010). Sulaimani Statistical Directorate. Summary of the preliminary results of the general population census for urban and rural residents of Sulaimani province in December 2009 by district, unpublished data.
44. Iran Data Portal. (2016). The Statistical Center of Iran. Population and Households by Provinces and Cities, Census 2016.
45. Chidamba, L., Cilliers, E., & Bezuidenhout, C. C. (2016). Spatial and temporal variations in pollution indicator bacteria in the Lower Vaal River, South Africa. Water Environment Research. 88(11), 2142-2149.