



Effect of seasonal variations in the microbiological quality of water (a Case study in Ahar River and Sattar Khan Dam of Iran)

Javad Eslami Khosrowshahi^{1*}, Mammad Ahad Oglu Salmanov¹

¹ Institute of Microbiology, National Academy of Sciences of Azerbaijan, Baku, Azerbaijan, E-mail: Javad_eslami35@yahoo.com

ABSTRACT



The quality of surface water is one of the most important concerns in many parts of the world. Regular analysis of surface water quality helps accurate management to reach acceptable quality for drinking water; however, its quality may be affected by seasonal variations. The aim of this study was to evaluate the microbiological quality of the water from the Ahar River and Sattarkhān dam (Iran) in different seasons. The physicochemical properties and the count of saprophytic, ammonifying, denitrifying, nitrite-fixing (aerobic and anaerobic), and coliforms were investigated in the samples during different seasons of a year. For this purpose, 4 stations in the river and 9 stations in the dam were selected for sampling. The mean count of saprophytic bacteria in the water of the River varied between 2.76 log CFU/ml (winter) and 4.66 log CFU/ml (fall). Mean total coliforms significantly differed ($p \leq 0.05$) among different seasons. At the entrance region of river water to the dam, the mean count of saprophytic bacteria and coliforms generally was more than it was in other stations. The distribution of ammonifying bacteria in the water of the dam was similar to saprophytes. The highest mean count of nitrogen-fixing and denitrifying bacteria was detected in the summer season. The conditions of the Sattarkhān dam were more favorable to *Azotobacter* sp. than those of *Clostridium pasteurianum*. The results of the present study indicated that seasonal variations and physicochemical parameters of the water of River and dam are directly or indirectly affect the levels of indicator bacteria. So, regular monitoring of rivers during different seasons of the year can lead to better water quality for human consumption and ultimately improve public health.

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* **Contact:** Javad Eslami Khosrowshahi  *Institute of Microbiology, National Academy of Sciences of Azerbaijan, Baku, Azerbaijan*,  Javadeslami35@yahoo.com

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INTRODUCTION

Nowadays, the quality of surface water has become one of the most important concerns in many parts of the world (Naddafi et al., 2007; Kumar et al., 2015; Taoufik et al., 2017). The quality of surface or ground waters can be affected by natural (geologic) and unnatural (pollution) factors (Karbassi and Pazoki, 2015; Kumar et al., 2015). Surface waters are more vulnerable to pollution than groundwaters due to the easy accessibility for the disposal of wastewater (Ogubanjo and Rolajo 2004; Taoufik et al., 2017). Increasing urbanization, industrial and agricultural practices, and dam construction have negative impacts on the quality of surface waters, especially in developing countries. In most countries, consumption of microbiologically polluted water contributed to the principal risks to human health (Kolawole et al., 2011). Thus, the microbial examination of water is crucial for proper water management to reach acceptable quality for drinking water (Kolarević et al., 2011; Mirzaei and Hasanian, 2013).

In Middle East countries such as Iran, there are few studies involved in the monitoring of the microbiological quality of rivers (Shahsavaripour et al., 2012; Fatemi et al., 2016; Besharati et al., 2018). Ahar River (Ahar Chāy), located in East Azerbāijān province of Iran, is one of the main sources of supplying fresh water for the inhabitants of this region. Sattārkhān Dam is built along the River in 1996 to meet the needs of Ahar city and surrounding villages to freshwater, optimal use of water, prevention of floods. Ahar River is a distinct ecosystem due to its climatic conditions; heavy rainfall in spring and fall, as well as the melting of snow leftover from the winter. The water of the river is likely to be contaminated with compounds of external origin during the rainy months of the year. This contamination may also occur during the summer when livestock continually uses the water of the river.

Therefore, the present study was aimed to evaluate the microbiological quality of water in Ahar River as well as in Sattārkhān dam and investigate the anthropogenic effects during different seasons of the year.

METHODS

Study area

Ahar River was chosen for this research. It is located the upstream of Sattārkhān dam at the west of Ahar city. This River (Ahar Chāy) stretches over a length of 240 km, originating from the Peer Saghā Mountain, runs in the east direction after passing through the south of Ahar city. The Dam is located downstream of the river (38° 46' 67" N, 46° 87' 71" E). The dam with a height of 75 m, a crest length of 350 m, and a storage lake of 135×10⁶ m³;

supplies the drinking water for Ahar city and surrounding villages.

Sampling procedure

In this study, the water of the river and dam were sampled from different stations. For this purpose, 4 stations in the river (Dāshkasan, Varzaqān, Rashtābād, and Owrang) and 9 stations in the dam were selected for sampling (Figure 1). The sampling procedure was carried out according to the APHA method (APHA, 2012). Water samples were collected from 20-30 cm under the surface of the water using a clean sterilized bottle of 250mL capacity. Samples were taken over one year from the selected stations and transported immediately to the laboratory on ice and stored at 4 °C till the experiments (Traoré et al., 2016).

Measurement of physicochemical parameters

Temperature, pH, dissolved oxygen (DO), and nutrients (NO₃⁻, NO₂⁻, NH₄⁺, and PO₄⁻) were measured using a portable multi-parametric probe (Hagh, USA) and UV-Vis Spectrophotometer (Aazami et al., 2015; Rice et al., 2017).

Microbiological analysis

Microbiological experiments were performed 2-3 hours after sampling. Enumeration of Saprophytic bacteria was carried out using the pour plate method (APHA, 1989). The nutrient agar (Merck, Germany) supplemented with glucose and yeast extract (5 and 3 g/l) was used for this purpose (El-Shenawy et al., 2005). The count of *Azotobacter* sp. fixing atmospheric nitrogen under aerobic conditions (CFU/ml) was calculated after 48-72h of incubation at 28°C on nitrogen-free Jensen's medium (Tripathy and Ayyappan, 2005). The count of *Clostridium pasteurianum* (*C. pasteurianum*) fixing atmospheric nitrogen under anaerobic conditions (MPN/100 ml) was also enumerated using Winogradsky's medium after 7 days of incubation at 25°C (Gołaś et al., 2008). Ammonifying and denitrifying bacteria were cultured in peptone ammoniation medium and denitrifying bacteria culture medium, respectively (Rodina, 1972). Quantification of total coliforms was performed using Eosin-methylene blue agar (Merck, Germany) after incubation at 37°C for 24 h (Kolarević et al., 2011).

Data analysis

To compare the means of data, analysis of variance (One-way ANOVA) was performed at the significance level of 95% using SPSS software (Version 23.0).

RESULTS AND DISCUSSION

For the first time, a microbiological study was performed on one of the most important

freshwater resources in Iran. Considering the economic, geographical, and social importance of the area, 4 stations in the Ahar River and 9 stations in the Sattarkhān dam were selected for sampling. Since external interactions in these stations are likely to be high, they were selected for sampling in the present study.

The sampling stations and the mean levels of selected water quality parameters of Ahar River and Sattarkhan Dam in the study period during different seasons are summarized in Table 1 and Table 2, respectively. The results of the present study showed that the levels of selected water quality parameters in the Ahar River changed based on different seasons and geographical locations during the study period. In most samples, the microbiological properties of water were changed according to its physicochemical parameters. The physicochemical indicators of water quality have seasonal variations. The water of the river and dam was mildly alkaline. The pH level was varied between 8.13 in Dāshkasan (spring) and 8.65 in Varzaqān (winter); however, significant differences were not observed between the pH levels in different stations ($p > 0.05$). The pH values were increased towards downstream water of Ahar River. Similar results are reported by Soo et al. (2014) in Malaysia.

The mean level of DO was higher in winter than in other seasons. Its level was reduced during summer that can be a result of the reduction in rainfall and increase in the salts content of water. By increasing the temperature of water in summer, the activity of microorganisms will be increased that result in the reduction of DO content (Besharati et al., 2018).

During the year, the concentration of nitrite and nitrate was at a satisfactory level (WHO, 2011; EC 91/676/EEC, 2018). However the ammonium and phosphate content was higher than recommended levels (WHO, 1996a; WHO, 1996b; Fadiran et al., 2008). Dāshkasan station had the significantly ($P < 0.05$) lowest mean of NH_4^+ (0.45), NO_3^- (2.27), and Phosphate (0.11). Since the position of this station is in the upstream part of the river, lower levels of agricultural effluents and sewage may be enters the water. Khoramnejadian and Fatemi (2016) also have reported similar results about the Damavand River.

Saprophytic bacteria can often cause reservoir souring and serious corrosion in water pipes and facilities (Qia et al., 2018). The count of saprophytes could be affected by season, salinity, organic nutrients, and geography (Thayib et al., 1989; Kuznetsov, 2012). In the water of the Ahar river, changes in the saprophytic bacterial count during different seasons were statistically significant ($p < 0.05$) (Table 3). The mean count of saprophytes varied between 2.73 log CFU/ml

(winter) and 4.42 log CFU/ml (fall). During the months with a low level of rainfall (winter), the count of saprophytes was ranged from 2.69 to 3.04 log CFU/mL. Hence, these values were increased to 4.36-4.57 log CFU/mL in spring. The increase of saprophytic bacterial count in spring may be due to the floods and rainfall and the erosion of the soil surface. The increase in their counts in summer may also be associated with the higher temperature of water in this season than others.

Since most plants and microorganisms in the water can't directly use the nitrogenous organic matter, these matters are degraded into absorbable components by microorganisms. Degradation of organic matter results in the formation of ammonia through the ammoniation process or oxidized to nitrite via the nitrification process (LeChevallier, 2003). In the samples taken from the Ahar River, the count of ammonifying was also studied. The variations in the count of ammonifying bacteria in different seasons of the year (except the winter) were not significant ($p > 0.05$) (Table 4). The mean count of these bacteria was ranged from 2.41 (winter) to 3.88 log CFU/ml (fall). The lowest mean count of ammonifying bacteria during different seasons was observed at Varzaqān and Dāshkasan (3.17 log CFU/ml). Hence, the highest mean count of those bacteria was detected at the Owrang station (3.66 log CFU/ml). The count of ammonifying bacteria in all the sampling sites was generally similar and stood at 10^3 CFU/ml (except the winter). Statistical analysis did not show a significant difference between the counts of these bacteria in different sampling sites ($p > 0.05$).

Rivers and dams are considered the main surface water resources in the rural areas of developing countries (Kuta et al., 2014; Muhammad et al., 2017). Because of their open exposure to the environment, these resources are susceptible to different contaminants (Gangil et al., 2013; Oludairo and Aiyedun, 2016; Muhammad et al., 2017). Contamination of Surface water resources is associated with farming, sewage discharge, and the presence of animals next to rivers. The animal's urination and defecation in surface water sources can result in high levels of coliforms (Kolarević et al., 2011; Afsatou et al., 2016). Due to the presence of several villages and pastures along the Ahar River, agricultural activities like livestock grazing and farming in the area probably contributed to the contamination of water to coliforms. The mean Coliforms counts in the water of River in different seasons of the year are shown in Table 5. All samples tested in the present study were positive for coliforms. Concentrations of total coliforms in the Ahar river samples were higher than limits regarded as safe for drinking water by Iranian national standards (0 CFU/100ml),

indicating the extent of contamination of the water sources making them unsafe for food processing and drinking. Such levels of contamination can threaten public health, and the use of contaminated water for drinking and food processing may lead to waterborne infections in the region. The mean total coliform count significantly was different ($p \leq 0.05$) during the seasons. Like other microbial groups, the higher counts of coliforms were detected in the spring and fall seasons (Table 5). The Owrang station was found to be the most contaminated sampling point with the highest total coliform count of 388.1 CFU/100ml in spring. High counts of coliform bacteria in downstream stations (Owrang) are probably associated with runoff flows in the upstream and sewage discharged from surrounding villages and livestock farms. Similar results have also been reported by Kirschner et al. (2014) and Khoramnejadian and Fatemi (2016). The mean counts of total coliforms in the stations were above the WHO limits of zero count/100 ml (WHO, 2011).

Due to the strategic importance of the Sattārkhān dam, the study on the microbiological quality of dam water was the main objective of this study. The changes in the saprophytic bacterial count in dam water were not significant at different seasons (except winter) ($p > 0.05$) (Table 6). Like the Ahar River, the total count of saprophytes in the winter was about 1 log CFU/ml lower than it was in other seasons. Furthermore, the count of saprophytes in the entrance area (A_1 , A_2 , and A_3) and along the shores of the dam was more than its central areas. This phenomenon may be related to contamination of entering the water to organic or inorganic nutrients of external origin. In the samples from the shores of Dam, due to soil erosion by water waves, large amounts of soil microbes can release into the water of the dam. Considering the variations of water temperature from winter (2.6-4.2 °C) to summer (21.0-23.4 °C), it can be concluded that by increasing the temperature of the water, the count of saprophytes was also increased. Hence, the effect of the season (mainly temperature) and location (station) on the count of saprophytes was evident.

Microbial communities in the aquatic ecosystems play an important role in nutrient recycling which includes nitrogen fixation, ammonification, nitrification, and denitrification processes (Zhao et al., 2015). Ammonifying and denitrifying bacteria can ammonify and denitrify which can strongly affect water quality (Herbert, 1982; Zhao et al., 2015). The presence of excess nitrogen in aquatic ecosystems can change the ecosystem structure and function and decreased the water quality. As a result of eutrophication of water with nitrate and nitrite, the growth of planktonic primary

producers can also be increased and this has considerable effects on the quality of water (Zhao et al., 2015). The count of ammonifying bacteria generally was lower than the saprophytic bacterial count in the dam water (Table 7). In different seasons, the distribution of ammonifying bacteria in the water of the dam was similar to saprophytic bacteria, and the lowest concentration of bacteria was detected in the winter. The count of ammonifying bacteria gradually increased from winter to summer (Table 7). It is reported that the growth of ammonifying bacteria is decreased at a low temperature of winter. However, it is markedly increased in spring followed by a release of ammonium into the water (Donnelly and Herbert, 1996; Poulin et al., 2007). In the present study, this trend was continued in summer. Due to the frequent rainfall and the increase of temperature in summer, water flow rates raised which results in an increased flow of organic matter containing nitrogen as the substrates for ammonifying bacteria into the surface water (George et al., 2004; Djuikom et al., 2006). Furthermore, there was a positive correlation between NH_4^+ and ammonifying bacteria. It is reported that high levels of ammonium can stimulate the growth of ammonifying bacteria (Yang et al., 2007).

Similar to the Ahar River, the pH of water in the dam was slightly alkaline during the year which is in agreement with the findings of Zhao et al. (2015) in lakes and reservoirs of the Northeast of China. The pH level is an important indicator of the growth of ammonifying and denitrifying bacteria (Venkatesharaju et al., 2010). Acidic or alkaline pH can inhibit the growth of ammonifying and denitrifying bacteria (Beverdorf et al., 2013). The highest value of pH in the water of the dam was recorded in the winter season with a mean level of 8.75. The lowest count of ammonifying and denitrifying bacteria was also detected in this season. Besides the temperature, the alkaline pH in winter may affect the microbial count.

DO was another significant indicator to affect microbial metabolism (Ahipathy and Puttaiah, 2006). Seasonal variations of DO for the dam are shown in Table 2. The highest and lowest levels of DO were recorded in winter and summer, respectively. During the summer, microorganisms consumed a considerable amount of oxygen along with the decomposition of organic matter. Ammonium and nitrite are the main nutrients in a water ecosystem (Zhao et al., 2015). Since the spring is the cultivation period along the Ahar River, the release of ammonium and nitrite to the river and consequently the dam was increased.

During the whole period of the present study, the count of *C. pasteurianum* did not exceed 70.3 MPN/100 ml, whereas those of *Azotobacter* sp.

cells were up to 120.5 CFU/ml (Table 8). This difference may be related to the thermal and oxygenation conditions, which were more favorable to aerobic nitrogen-fixing bacteria. These results are in agreement with the findings of Gołaś et al. (2008) in the Waters of the River Drwęca. The distribution of denitrifying bacteria was also similar to that of *C. pasteurianum* (Table 9). The distribution of denitrifying bacteria was changed during different seasons. The lowest count of them was recorded for winter when the dissolved oxygen was at maximum level. The negative correlation of denitrifying bacteria with DO was also reported by Zhao et al. (2015).

The total counts of coliform were evaluated in this study to assess the hygienic quality of accumulated water in the dam. It was found that this group of bacteria was in higher counts in the entrance area of the dam. In the central area and near the dam, the total coliform count was low in all seasons (Table 10). While the increase in water temperatures can have a positive effect on the count of microbes in summer, but the coliform count in summer was lower than those of spring and fall. The coliforms in the water of the river and dam may be related to the rainfall and soil erosion which is low in summer.

CONCLUSION

The results of the present study indicated that seasonal variations and physicochemical parameters of the water in Ahar River and Sattarkhan dam can affect the levels of saprophytic, ammonifying, denitrifying, nitrite-fixing bacteria and coliforms. Furthermore, the microbiological quality of water is directly or indirectly associated with the physicochemical parameters of water. The evaluated water samples in this study were contaminated with coliform to levels regarded as unsafe as drinking water or to use in food processing. Thus, to achieve acceptable water quality and compliance with national and international standards, monitoring the water quality and its disinfection at the point of use is necessary.

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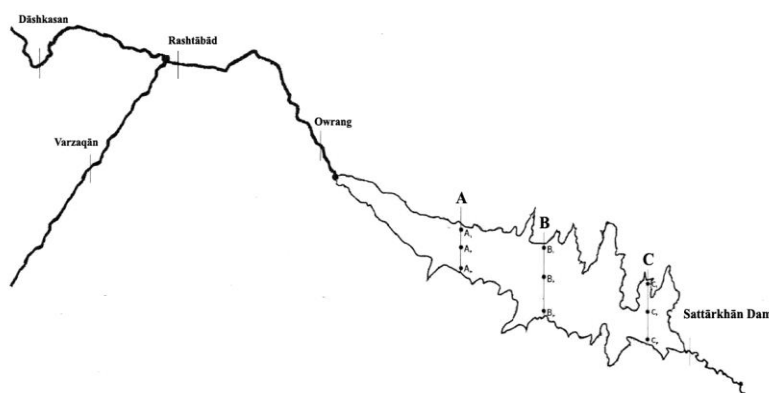


Fig. 1: Sampling stations of the Ahar River and Sattarkhān Dam

Table 1: The Mean levels of physicochemical parameters in the water of Ahar River during different seasons of 2019

Parameters	Station	Winter	Spring	Summer	Autumn
pH	Dāshkasan	8.53	8.38	8.42	8.44
	Varzaqān	8.21	8.50	8.56	8.49
	Rashtābād	8.37	8.16	8.54	8.24
	Owrang	8.60	8.13	8.65	8.29
T(°C)	Dāshkasan	4.1	16.1	23.3	11.1
	Varzaqān	1.8	13.4	20.5	11.3
	Rashtābād	3.2	15.2	22.8	13.4
	Owrang	2.7	14.6	20.4	12.7
Dissolved Oxygen (mg/l)	Dāshkasan	12.8	12.4	9.1	12.3
	Varzaqān	13.4	13.2	9.8	13.2
	Rashtābād	12.9	11.2	9.0	12.4
	Owrang	12.7	11.1	9.8	12.0
NH ₄ ⁺ (mg/l)	Dāshkasan	0.33	0.29	0.95	0.25
	Varzaqān	0.62	0.97	0.48	0.96
	Rashtābād	0.45	0.79	0.81	0.40
	Owrang	0.42	0.70	0.45	0.43
NO ₂ ⁻ (mg/l)	Dāshkasan	0.005	0.003	0.05	0.003
	Varzaqān	0.006	0.003	0.01	0.01
	Rashtābād	0.01	0.002	0.01	0.03
	Owrang	0.01	0.002	0.02	0.02
NO ₃ ⁻ (mg/l)	Dāshkasan	6.40	1.26	0.95	0.53
	Varzaqān	8.10	1.90	1.03	6.30
	Rashtābād	5.53	1.62	1.22	2.90
	Owrang	6.15	1.73	1.00	3.60
PO ₄ ⁻ (mg/l)	Dāshkasan	0.19	0.27	0.09	0.09
	Varzaqān	0.21	0.30	0.10	0.11
	Rashtābād	0.24	0.10	0.13	0.12
	Owrang	0.20	0.22	0.12	0.10

Table 2: The Mean levels of quality parameters in the water of Sattarkhan Dam during different seasons of 2019

	Station	Winter	Spring	Summer	Autumn
pH	A ₁	8.80	8.55	8.75	8.35
	A ₂	8.79	8.55	8.66	8.45
	A ₃	8.75	8.48	8.60	8.37
	B ₁	8.76	8.57	8.72	8.45
	B ₂	8.73	8.28	8.10	8.47
	B ₃	8.76	8.33	8.10	8.57
	C ₁	8.72	8.63	8.86	8.40
	C ₂	8.70	8.31	8.27	8.35
	C ₃	8.80	8.38	8.35	8.42
T(°C)	A ₁	2.6	16.2	23.4	7.8
	A ₂	3.1	15.6	22.1	7.4
	A ₃	3.3	14.8	21.6	6.9
	B ₁	4.2	16.4	22.1	8.4
	B ₂	3.7	15.9	23.5	8.5
	B ₃	3.5	13.4	22.8	7.9
	C ₁	3.4	14.9	21.5	8.5
	C ₂	3.8	12.7	22.9	8.4
	C ₃	3.4	13.4	21	8.3
Dissolved Oxygen (mg/l)	A ₁	10.9	10.2	9	10.4
	A ₂	11.3	11.3	9.2	9.8
	A ₃	11.5	10.6	10.3	9.7
	B ₁	10.4	10.3	9.6	10.3
	B ₂	11.9	11.2	8.5	10.2
	B ₃	11.4	11.4	8.4	10.6
	C ₁	10.9	10.8	8.6	11.1
	C ₂	10.8	9.3	11.6	10.6
	C ₃	11.7	10	11.4	10.9
NH₄⁺ (mg/l)	A ₁	0.22	0.33	0.91	0.29
	A ₂	0.41	0.48	0.89	0.67
	A ₃	0.39	0.45	0.76	0.50
	B ₁	0.38	0.51	0.58	0.49
	B ₂	0.29	0.40	0.85	0.64
	B ₃	0.47	0.61	0.79	0.68
	C ₁	0.38	0.50	0.75	0.69
	C ₂	0.34	0.59	0.67	0.60
	C ₃	0.26	0.39	0.84	0.72
NO₂⁻ (mg/l)	A ₁	0.15	0.39	0.06	0.42
	A ₂	0.16	0.23	0.04	0.38
	A ₃	0.15	0.26	0.01	0.30
	B ₁	0.1	0.23	0.00	0.29
	B ₂	0.15	0.23	0.00	0.32
	B ₃	0.22	0.32	0.00	0.20
	C ₁	0.19	0.26	0.00	0.23
	C ₂	0.19	0.20	0.00	0.36
	C ₃	0.11	0.18	0.00	0.29
NO₃⁻ (mg/l)	A ₁	3.1	5.5	0.5	2.8
	A ₂	2.7	3.2	0.0	3.1
	A ₃	2.4	3.3	0.0	2.5
	B ₁	2.3	2.3	0.0	2.7
	B ₂	1.4	2.8	0.0	1.9
	B ₃	1.3	2.7	0.0	1.9
	C ₁	1.4	3.4	0.0	1.8
	C ₂	3.1	4.4	0.0	2.6
	C ₃	3.0	4.2	0.1	2.5

PO₄⁻ (mg/l)	A ₁	0.17	0.25	0.01	0.31
	A ₂	0.24	0.26	0.01	0.20
	A ₃	0.09	0.16	0.00	0.14
	B ₁	0.15	0.23	0.00	0.16
	B ₂	0.05	0.10	0.00	0.13
	B ₃	0.19	0.22	0.00	0.12
	C ₁	0.24	0.30	0.00	0.14
	C ₂	0.25	0.26	0.00	0.12
	C ₃	0.10	0.19	0.00	0.10

Table 3: The count of saprophytic bacteria (Log₁₀ CFU/ml) in the water of Ahar River during different seasons of 2019

Station	Winter	Spring	Summer	Fall
Dāshkasan	2.78	4.36	4.04	4.56
Varzaqān	3.00	4.49	4.11	4.64
Rashtābād	2.48	4.58	4.20	4.68
Owring	2.70	4.36	4.26	4.76
Mean	2.74	4.45	4.15	4.66

Table 4: The variation in the count of ammonifying bacteria in the water of Ahar River (Log₁₀ CFU/ml) during different seasons of 2019

Station	Winter	Spring	Summer	Fall
Dāshkasan	2.40	3.42	3.46	3.41
Varzaqān	2.39	3.45	3.46	3.39
Rashtābād	2.41	3.44	3.47	4.32
Owring	2.42	4.40	3.44	4.40
Mean	2.41	3.68	3.46	3.88

Table 5: Total count of coliforms (CFU/100 ml) in the water of Ahar River during different seasons of 2019

Station	Winter	Spring	Summer	Fall
Dāshkasan	0.6	318.8	10.9	120.5
Varzaqān	2.6	198.9	20.5	185.4
Rashtābād	3.3	49.5	31.4	278.1
Owring	3.0	388.1	30.9	136.9
Mean	2.3	238.8	23.4	180.2

Table 6: Variations in the count of saprophytic bacteria (Log₁₀ CFU/ml) in the water of Sattārkhān Dam during different seasons of 2019

Station	Winter	Spring	Summer	Fall
A ₁	2.77	4.36	4.38	4.52
A ₂	3.57	4.20	4.22	4.41
A ₃	3.55	4.25	4.17	4.30
B ₁	3.63	4.14	4.13	4.25
B ₂	3.47	4.26	4.15	4.27
B ₃	3.68	4.26	4.16	4.27
C ₁	3.11	4.49	4.23	4.58
C ₂	3.25	4.06	4.25	4.24
C ₃	3.11	4.16	4.23	4.22
Mean	3.47	4.25	4.22	4.27

Table 7: The count of ammonifying bacteria (Log₁₀ CFU/ml) in the water of Sattarkhān Dam during different seasons of 2019

Station	Winter	Spring	Summer	Fall
A ₁	2.2	4.1	4.7	4.1
A ₂	2.1	4.5	4.9	4.3
A ₃	2.0	3.8	3.6	4.5
B ₁	2.6	4.5	4.3	4.0
B ₂	2.5	3.5	3.5	3.9
B ₃	2.1	4.6	4.1	3.5
C ₁	2.9	3.7	3.7	3.8
C ₂	2.7	3.5	3.9	3.5
C ₃	2.2	4.6	4.1	3.9
Mean	2.37	4.09	4.09	3.94

Table 8: The count of Bacteria fixing atmospheric nitrogen in the water of Sattarkhān dam during different seasons of 2019

Station	Azotobacter spp. (CFU/ml)				Clostridium pasteurianum (MPN/100 ml)			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
A ₁	5.2	10.4	5.2	7.3	0	3.1	11.5	31
A ₂	3.1	14.6	8.3	10.4	0.3	7.3	14.6	7.3
A ₃	4.1	20.9	30.1	11.4	0	9.4	19.8	9.6
B ₁	10.4	20.9	36.1	14.4	2.1	4.1	21.1	11.4
B ₂	6.2	18.8	41.2	18.6	3.1	6.2	37.1	18.7
B ₃	4.1	21.1	46.2	26.9	6.1	8.3	48.1	21.9
C ₁	5.2	18.8	56.2	24.1	8.3	12.5	57.2	26.7
C ₂	6.2	16.7	60.3	31.1	12.5	14.6	68.2	38.4
C ₃	5.2	18.8	120.5	36.2	10.4	16.7	70.3	44.1
Mean	5.52	17.89	44.90	20.04	4.76	9.13	38.66	23.23

Table 9: The count of denitrifying bacteria (CFU/ml) in the water of Sattarkhān dam during different seasons of 2019

Station	Winter	Spring	Summer	Autumn
A ₁	0.0	20.8	41.6	31.5
A ₂	0.0	31.5	62.1	31.7
A ₃	10.3	40.4	73.9	40.2
B ₁	28.1	62.9	90.1	60.3
B ₂	31.3	100.1	208.2	83.8
B ₃	0.3	104.5	209.4	100.7
C ₁	41.5	100.8	301.7	103.9
C ₂	31.6	104.4	502.1	109.4
C ₃	40.8	107.1	602.6	102.7
Mean	20.43	74.72	232.41	73.80

Table 10: Total count of coliforms (CFU/100 ml) of the water of Sattarkhān dam in different seasons of 2019

Station	Winter	Spring	Summer	Fall
A ₁	29.6	1540.7	320.1	110.4
A ₂	10.3	595.2	246.1	460.2
A ₃	21.2	328.1	125.5	175.8
B ₁	14.8	420.1	130.6	200.9
B ₂	6.9	186.8	96.4	110.5
B ₃	8.5	95.4	50.2	193.9
C ₁	7.8	85.4	55.2	140.6
C ₂	5.9	72.3	40.1	126.5
C ₃	9.7	115.5	100.4	120.4
Mean	12.7	382.1	129.4	182.13