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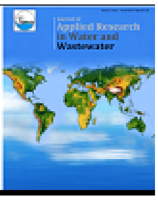

Spatial distribution of indicator bacteria in Khersan-3 dam waters, Lordegan County, Southwestern Iran

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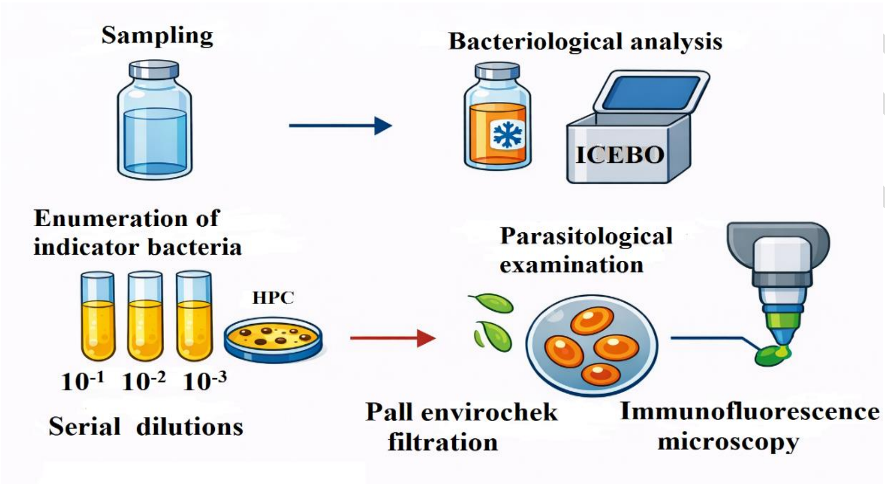
GRAPHICAL ABSTRACT



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ABSTRACT

The Khersan River, the largest tributary of the Karun River, is regulated by the Khersan-3 Dam, constructed for flood control, irrigation management, and hydropower generation. Despite its strategic importance, no comprehensive study has previously assessed microbial water quality in this reservoir, particularly under the pressure of intensive agricultural activities in the watershed. This study investigated heterotrophic bacteria, total and fecal coliforms, and protozoan (*Giardia* and *Cryptosporidium*) contamination in the Khersan-3 sub-basin. Monthly sampling was carried out from November 2023 to October 2024 at five stations using sterile glass bottles (100 ml) from the surface layer (20 cm depth). For protozoan detection, 10 liter samples were collected at Station 5. Results showed seasonal and spatial variations in microbial indicators. The highest mean concentration of heterotrophic bacteria was observed in autumn 2023 (6.237 Log CFU/100 ml), while the lowest occurred in summer 2024 (4.507 Log CFU/100 ml) ($p<0.05$). Total coliforms reached their maximum in winter 2023 (3.822 Log CFU/100 ml) and decreased to a minimum of 2.509 Log CFU/100 ml in summer 2024 ($p<0.05$). However, seasonal variations in the mean density of fecal coliforms were not statistically significant ($p>0.05$). Across stations, the densities of heterotrophs, total coliforms, and fecal coliforms did not differ significantly ($p>0.05$). *Cryptosporidium* was undetected in all samples, whereas no significant difference was found in *Giardia* occurrence among seasons, despite detection in approximately 40% of spring samples ($p>0.05$). This study provides the first evidence of substantial temporal and spatial fluctuations in indicator bacteria across the Khersan-3 Dam watershed, highlighting the influence of hydrological and anthropogenic drivers. The findings emphasize the need for targeted management actions, including continuous microbial surveillance, stricter control of agricultural runoff, and improved water treatment practices to safeguard public health and aquaculture sustainability.

1. Introduction

Freshwater resources, particularly rivers and dam reservoirs, play a crucial role in providing drinking water, supporting agriculture, generating energy, and sustaining regional ecosystems. Dams, by collecting and regulating rainfall runoff, represent key infrastructures for water resource management. However, the quality of stored water in reservoirs is influenced by both natural and human-induced factors,

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such as climatic variations, contaminated rainfall, domestic waste, and agricultural or industrial discharges (Albaggar, 2021; Akhtar *et al*., 2021).

Human activities including agriculture, wastewater discharge, mining, and construction introduce pesticides, heavy metals, and microbial pathogens into aquatic systems, threatening human health and ecosystem balance (Caravello *et al*., 2007). In this context, biological indicators such as coliforms, enterococci, and heterotrophic bacteria are widely used for monitoring microbial water quality. These microorganisms are highly responsive to environmental changes and are directly associated with fecal contamination, making them reliable markers for assessing the sanitary status of water resources (Bhaduri *et al*., 2022; Wen *et al*., 2020). Several studies have confirmed that the presence of such bacteria in reservoir waters may reflect inputs of human or animal waste (Chen *et al*., 2018; Kristanti *et al*., 2022).

Heterotrophic bacteria, which depend on organic carbon sources for growth, tend to proliferate under unfavorable conditions such as elevated temperatures, inappropriate pH, or reduced residual chlorine, thereby diminishing microbial water quality (Banu *et al*., 2021; Ansa *et al*., 2017). Similarly, fecal coliforms such as *Escherichia coli*, *Klebsiella, Enterobacter*, and *Citrobacter* are classical indicators of fecal pollution and are often linked with antibiotic resistance (Quarcoo *et al*., 2022).

In addition to bacteria, protozoan parasites such as *Cryptosporidium* and *Giardia* are significant waterborne pathogens transmitted through contaminated water or food. These organisms cause diseases such as cryptosporidiosis and giardiasis and pose particular risks to children, the elderly, and immunocompromised individuals. Their resistance to conventional disinfection methods, including chlorination, presents major challenges to water treatment systems (Adeyemo *et al*., 2019; Maxamhud *et al*., 2025).

With the growing importance of accurate water monitoring, molecular techniques such as quantitative PCR (qPCR) and metagenomics have facilitated faster and more precise identification of microbial and protozoan indicators (Wang *et al*., 2025). When integrated with physical and chemical data, these tools support a holistic approach to sustainable water resource management (Choix, Palacios and Nevarez-Moorillón, 2023).

The Khersan-3 Dam, located in Lordegan County, Chaharmahal and Bakhtiari Province, is a double-arch concrete dam designed for flood control, hydropower generation, and aquaculture development. Despite its strategic role, microbial water quality in this reservoir has not been comprehensively studied, creating a clear research gap.
Therefore, microbial assessment of the Khersan-3 Dam water, particularly focusing on indicator bacteria, coliforms, heterotrophic bacteria, and protozoa such as *Giardia* and *Cryptosporidium*, is crucial for evaluating the sanitary and ecological health of the reservoir.

2. Materials and methods

2.1. Sampling stations

The geographical locations of the sampling stations are presented in Table 1, and their spatial distribution is shown in Figure 1. The five sampling stations were selected to capture Changes in potential pollution sources across the Khersan-3 Dam watershed. Two stations on the Marbareh River (near Ab Malakh and Narmeh villages) represent rural and livestock influences, while the Bashar River station reflects possible industrial impacts. The confluence of the Bashar and Marbareh rivers was chosen to assess combined flow and pollution load, and the upstream Khersan River station serves as a reference point before reservoir inflow. All analyses were performed in triplicate to ensure accuracy and reproducibility.

Table 1. Location of sampling stations in the Khersan-3 Dam watershed.

Station no.	Station name	Geographical coordinates (UTM)
1	Marbareh River (under the Ab-Malakh village bridge)	X=535704, Y=3445852
2	Marbareh River (before the Narmeh village bridge)	X=515763, Y=3445728
3	Bashar River (downstream of the West Iran Gas Pressure Station)	X=521484, Y=3432621
4	Khersan River (after confluence of Bashar and Marbareh Rivers)	X=513880, Y=3442381
5	Khersan River (before Khersan-3 Dam construction site)	X=499030, Y=3456600

2.2. Bacteriological analysis

Water samples were collected in pre-sterilized polyethylene bottles and transported to the laboratory in iceboxes at 4 °C to prevent microbial alteration. All analyses were performed immediately upon arrival. Bacterial enumeration was conducted using the plate count method, which estimates the number of viable bacterial colonies capable of growth on a specific nutrient medium (Bharathi *et al*., 2018).

For accurate colony estimation, samples were serially diluted to achieve a countable range of 30–300 colony-forming units (CFU) per plate. Colony counts below 30 were considered statistically unreliable; therefore, serial dilutions were performed to obtain valid results (Shehata *et al*., 2025).

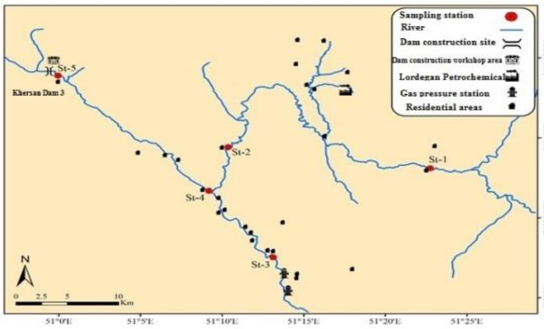

Fig. 1. Map of study stations in the rivers under the Khersan 3 Dam basin.

2.3. Serial dilution procedure

Approximately 1 ml of each water sample was transferred into a sterile test tube containing 9 ml of distilled water to obtain a 10^{-1} dilution. From this, 1 ml was pipetted into another sterile tube containing 9 ml of distilled water to produce a 10^{-2} dilution, and the process was repeated sequentially to prepare a 10^{-3} dilution (Barik and Biswal, 2024).

2.4. Enumeration of indicator bacteria

Heterotrophic bacteria were quantified using the Heterotrophic Plate Count (HPC) method on R2A agar. Plates were incubated at 37 °C for 48 hours (APHA, 2017). Total and fecal coliforms were enumerated using ECC chromogenic agar. Incubation was carried out at 37 °C for total coliforms and 44 °C for fecal coliforms for 48 hours, respectively (Murei, Kamika and Momba, 2024).

2.5. Logarithmic normalization of fecal coliform

To normalize the data and reduce variability in the measured values of fecal coliforms, raw counts expressed as CFU/100 ml were transformed to a logarithmic scale. The transformation was performed using base-10 logarithms according to Eq. 1 (APHA, 2017):

$$\text{Log } 10(N) \tag{1}$$

where, N represents the number of colony-forming units per 100 ml of water sample. Logarithmic transformation is a common approach in water quality studies, as it reduces data heterogeneity and enhances the reliability of statistical analyses (Tallon *et al*., 2005).

Following transformation, the log-transformed values were analyzed using Microsoft Excel. To evaluate the statistical behavior of the data, boxplots were constructed, displaying median, mean (×), first quartile (Q1), third quartile (Q3), and minimum and maximum values. The interquartile range (IQR) was calculated using Eq. 2:

$$IQR = Q3 - Q1 \tag{2}$$

Acceptable data ranges were then determined based on the 1.5×IQR criterion, as expressed in Eqs. 3 and 4:

$$\text{Lower bound} = Q1 - 1.5 \times IQR \tag{3}$$
$$\text{Upper bound} = Q3 + 1.5 \times IQR \tag{4}$$

Data points falling outside these ranges were identified as outliers. The results indicated that all log-transformed values were within the defined range, with no outliers detected, confirming the effectiveness of logarithmic transformation in reducing initial variability and improving the dataset's statistical distribution.

2.6. Parasitological examination

Detection of *Giardia* and *Cryptosporidium* cysts and oocysts was performed using Pall Envirochek filtration followed by immunofluorescence microscopy (ISIRI, 2014).

2.7. Microbial quality standards

Table 2 summarizes the key bacterial indicators used for water quality assessment based on the Iranian National Standard (ISIRI, 2014) and international criteria (WHO/EPA). The threshold values serve as reference limits for determining the microbiological safety of Agricultural, surface, and recreational waters.

Table 2. Permissible thresholds for microbial indicators in water.

Microbial / parasitological indicator	Agricultural water limit (FAO / WHO)	Surface / recreational water limit	Water type	Explanation
Heterotrophic Bacteria (HPC)	Usually no specific standard	Typically not regulated	Agricultural / Surface water	Mostly used for drinking water monitoring
Total Coliforms	≤1000 CFU/100 ml (general guideline)	≤200 CFU/100 ml	Surface / Recreational water	Indicates environmental contamination
Fecal Coliforms	≤1000 CFU/100 ml	≤200–400 CFU/100 ml	Agricultural/ Surface water	Indicator of fecal contamination
Escherichia coli (*E. coli*)	≤1000 CFU/100 ml (non-food crop irrigation)	≤126 CFU/100 ml (geometric mean)	Agricultural / Surface water	Most important health indicator for irrigation water
Giardia cysts	No specific requirement	Occasional monitoring	Surface water	Mostly examined in raw water sources
Cryptosporidium oocysts	No specific requirement	Occasional monitoring	Surface water	Important in raw water sources

2.8. Statistical analysis

Data normality was assessed using the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) and Duncan's multiple range test were applied to compare mean values of microbial parameters across sampling sites, months, and seasons. Statistical significance was considered at $p<0.05$. All analyses were conducted using SPSS software (version 18). Experiments were performed in triplicate, and qualitative data (e.g., *Giardia* presence/absence) were analyzed using non-parametric tests.

3. Results and discussion

3.1. Monthly logarithmic variations of bacterial indicators

The monthly logarithmic variations of heterotrophic bacteria, total coliforms, and fecal coliforms during 2023–2024 exhibited notable temporal dynamics (Fig. 2). Significant differences were observed in heterotrophic bacteria, with higher concentrations in December (6.75 Log CFU/100 ml) compared to March (4.15 Log CFU/100 ml) ($p<0.05$). Total coliforms also showed significant variation, being greater in February (4.56 Log CFU/100 ml) than in September (1.93 Log CFU/100 ml) ($p<0.05$). In contrast, monthly variations in fecal coliforms did not reveal any statistically significant differences ($p>0.05$). These statistically significant fluctuations highlight seasonal variations in microbial load, which are likely influenced by climatic conditions, rainfall patterns, temperature fluctuations, and human activities. The decline in heterotrophic bacterial density observed in March may be linked to reductions in dissolved organic matter and lower water temperatures, consistent with Cheng *et al*. (2024), who reported that low temperatures and decreased dissolved oxygen reduce microbial diversity in European surface waters. The peak in total coliforms in February may reflect the stabilizing effect of lower temperatures on bacterial populations, similar to findings by Zamorska, Karwowska and Przystaś (2023), who demonstrated that microbial indicators remain more stable under cold conditions. The increased fecal coliform concentrations in June are likely driven by higher temperatures and agricultural runoff during the plant growing season, aligning with Locke *et al*. (2024), who highlighted the influence of seasonal rainfall and agricultural activities on bacterial community composition in rivers feeding Lake Taihu. Moreover, the pronounced fluctuations in fecal coliform densities from May to September suggest a direct impact of human activities, raw wastewater discharge, and climate variability on microbial water quality. Comparable seasonal increases in fecal indicators have been reported by Fulke, Mamidala and Nikalje (2025) in the Persian Gulf, particularly in areas with high anthropogenic pollution, reinforcing the regional relevance of our findings.

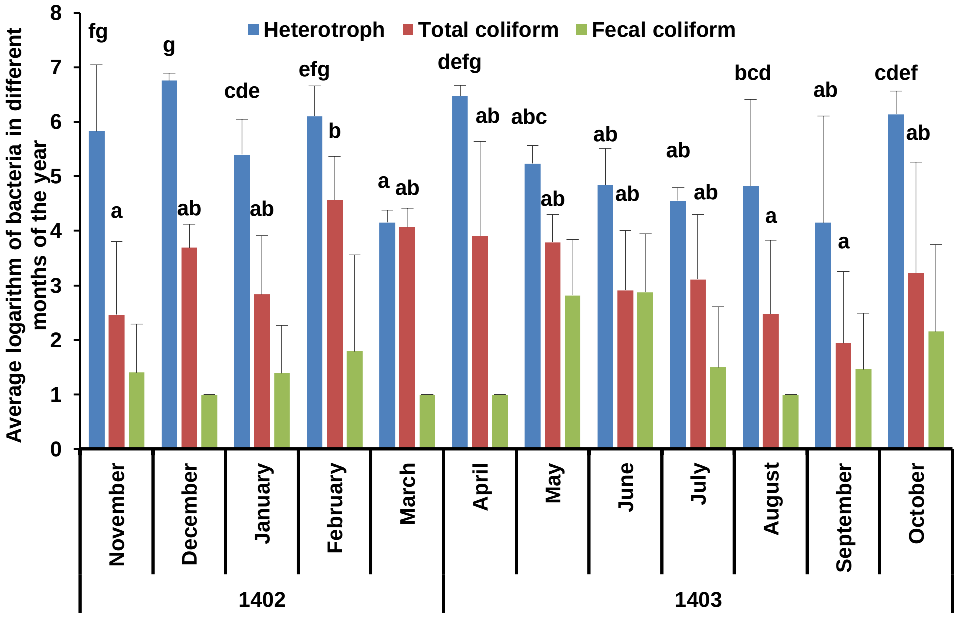

Fig. 2. Monthly logarithmic variations of heterotrophic bacteria, total coliforms, and fecal coliforms (CFU/100 ml) in the Khersan 3 sub-basin (Different letters in each column indicate significant differences among the data Indicators without letters showed no significant differences).

3.2. Logarithmic distribution of fecal coliforms

The boxplot analysis of fecal coliforms demonstrated a median value of approximately 1, suggesting the predominance of moderate concentrations during most months. The first (Q1) and third (Q3) quartiles were calculated as 0.7 and 2.3, respectively, resulting in an interquartile range (IQR) of 1.6. Based on the 1.5×IQR criterion, the acceptable data range extended from 1.7 to 4.7, with no outliers detected. The whiskers ranged from a minimum of 0.3 to a maximum of 3, and the mean (×) was positioned near the box center. These results indicate that logarithmic transformation effectively minimized initial data dispersion and produced a more balanced and normal-like distribution (Fig. 3).

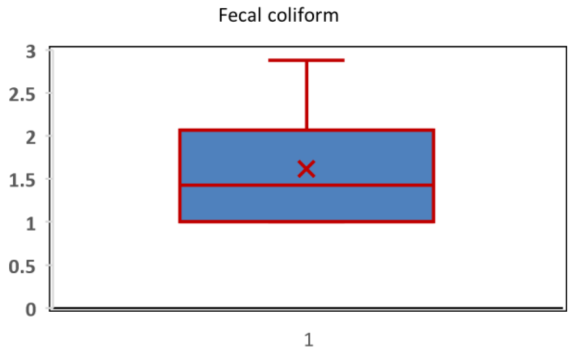

Fig. 3. Boxplot showing monthly logarithmic variations in fecal coliforms (CFU/100 ml) in the Khersan 3 sub-basin.

Logarithmic transformation of fecal coliform data in this study effectively reduced skewness and extreme dispersion in the raw dataset, resulting in a more compact distribution in the boxplot. This approach is particularly suitable for microbial datasets, which often exhibit very high values and pronounced seasonal fluctuations, as it stabilizes variance and approximates a normal distribution (Herrig *et al.*, 2019; West, 2022). The absence of outliers after transformation indicates successful normalization and allows the application of classical statistical tests such as ANOVA (Cude, 2005).

3.3. Seasonal logarithmic variations of bacterial indicators

Seasonal monitoring revealed the influence of environmental and climatic factors on microbial abundance in surface waters. Significant differences were observed in heterotrophic bacteria, with higher mean concentrations in autumn 2023 (6.237 Log CFU/100 ml) compared to summer 2024 (4.507 Log CFU/100 ml) ($p<0.05$). Total coliforms also showed significant variation, being greater in winter 2023 (3.822 Log CFU/100 ml) than in summer 2024 (2.509 Log CFU/100 ml) ($p<0.05$). However, seasonal variations in the mean density of fecal coliforms did not show any significant differences ($p>0.05$) (Fig. 4).

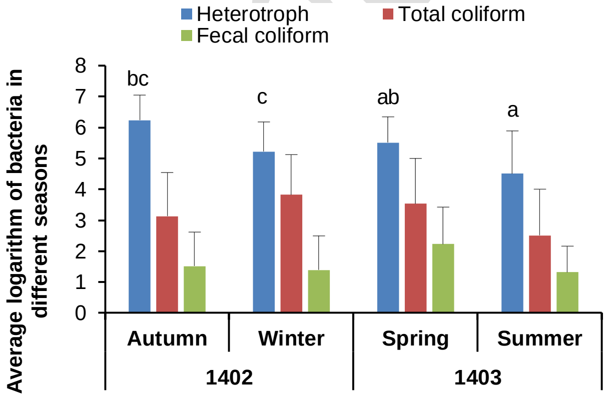

Fig. 4. Seasonal logarithmic variations of heterotrophic bacteria, total coliforms, and fecal coliforms (CFU/100 ml) in the Khersan 3 sub-basin during 2023–2024 (Different letters in each column indicate significant differences among the data Indicators without letters showed no significant differences).

Seasonal analysis revealed statistically significant fluctuations in microbial indicators ($p<0.05$, Duncan's multiple range test). Autumn exhibited the highest heterotrophic bacterial densities, likely influenced by the input of organic matter from early rainfall and surface runoff, while summer showed the lowest levels, reflecting the combined effects of high temperatures, solar radiation, and reduced pollutant inputs on microbial survival. These mechanistic patterns are consistent with the findings of Tagar *et al.* (2025), who reported higher microbial loads during the wet season compared to the dry season in the Indus River. Similarly, Herrig *et al.* (2019) observed enhanced fecal indicators with increased rainfall and river discharge in the Rhine and Mosel rivers, while elevated temperatures and solar radiation reduced them. Maipa, Alamanos and Bezirtzoglou (2001) reported summer increases in bacterial indicators, including *E. coli* and fecal coliforms, along Mediterranean coasts in Greece, attributed to tourism pressure and increased wastewater discharge. Overall, variations between the present study and previous reports likely reflect differences in climate, land use, and the intensity of human activities in the study region. This discussion emphasizes mechanistic explanations supported by statistically significant results and relevant regional studies, thereby avoiding overly speculative interpretations.

3.4. Stationary logarithmic changes of bacterial populations

Stationary analysis revealed no statistically significant differences in bacterial abundance across sampling stations ($p>0.05$). For heterotrophic bacteria, concentrations among stations did not differ significantly ($p>0.05$). Total coliforms also showed no significant variation across stations ($p>0.05$). Similarly, fecal coliforms exhibited no statistically significant differences among stations ($p>0.05$) (Fig. 5).

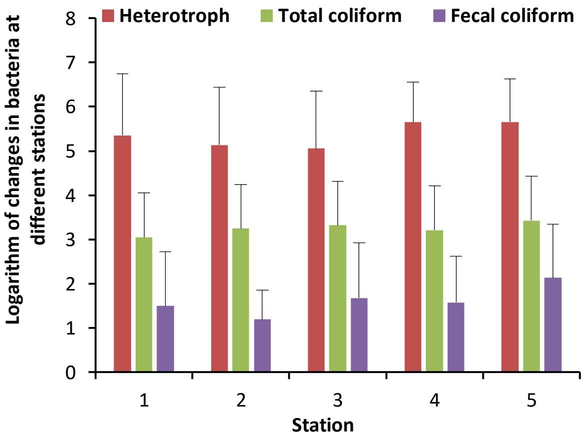

Fig. 5. Stationary logarithmic variations of heterotrophic bacteria, total coliforms, and fecal coliforms (CFU/100 ml) across sampling stations in the Khersan 3 sub-basin (Indicators without letters showed no significant differences).

These findings indicate spatial heterogeneity in microbial contamination, reflecting variations in local pollution sources as well as broader watershed characteristics. Although downstream stations (particularly Stations 4 and 5) are located in areas receiving cumulative discharges from upstream settlements, agricultural fields, and small industries, statistical analysis did not reveal significant differences in microbial abundance among stations ($p>0.05$). The observed numerical variations may therefore reflect local pollution pressures, but these were not statistically significant. In contrast, upstream stations with lower human activity exhibited reduced contamination levels. Moreover, hydrological connectivity including flow direction, runoff pathways, and convergence of tributaries appears to facilitate the downstream transport and accumulation of microbial pollutants. Stationary analysis further indicated that bacterial indicators are simultaneously influenced by both location and seasonal variation. The interaction of hydrological processes with seasonal rainfall patterns may enhance microbial transport during wetter periods, whereas reduced flow during dry seasons limits dispersion but may concentrate pollutants in localized zones. These spatial patterns are consistent with observations in similar systems. Hong *et al.* (2025) reported that fecal coliform concentrations in South Korea's Nakdong River increased significantly at downstream stations during warmer seasons and were closely associated with cumulative rainfall and wastewater treatment efficiency.

Likewise, Muñoz-Delgado *et al.* (2025) found that downstream stations with high discharge and urban wastewater inputs exhibited the highest fecal coliform concentrations in the Santiago–Guadalajara River basin. Bharathi *et al.* (2018) observed positive correlations between heterotrophic bacteria, coliforms, and organic load (BOD), as well as nutrient concentrations, along the Ennore coast in India, with higher bacterial growth in coastal stations and warmer seasons. Calheiros *et al*. (2021) reported that seasonal changes and vegetation structure significantly affected the removal of fecal indicators in a constructed wetland in Portugal, with lower bacterial removal efficiency during summer.

3.5. Parasitological parameters

Cryptosporidium oocysts were not detected throughout the study, though this may reflect concentrations below the method's detection limit rather than absolute absence. In contrast, *Giardia* cysts were intermittently observed, particularly during spring, but non-parametric analyses indicated no statistically significant interannual (Mann–Whitney U = 13.5, p = 0.428), monthly (Kruskal–Wallis χ^2 = 11.0, p = 0.443), or seasonal differences (χ^2 = 2.75, p = 0.432) (p>0.05) (Table 2).

Table 3. Biological (parasitological) parameters (Total/100 ml) at Station 5 of the Khersan 3 sub-basin.

Parameter	November	December	January	February	March	April	May	June	July	August	September	October
Total/100 ml *Cryptosporidium*	–	–	–	–	–	–	–	–	–	–	–	–
Total/100ml *Giardia*	–	–	–	+	–	+	+	+	–	–	–	+

The occurrence of *Giardia* is likely influenced by hydrological and land-use factors, such as rainfall-driven runoff, livestock activity, and wastewater inputs, while the absence of *Cryptosporidium* may result from lower environmental persistence or limited contamination sources. Similar studies have reported comparable findings. Ongerth and Karanis (2018) noted that rainfall and livestock activity strongly contribute to *Giardia* contamination in surface waters, while *Cryptosporidium* often shows lower detection rates due to reduced persistence. In addition, Efstratiou, Ongerth, J.E. and Karanis (2017) highlighted that *Giardia* is more frequently detected than *Cryptosporidium* in European water supplies, supporting the idea of differential survival and contamination sources.

4. Conclusions

This study demonstrates that bacterial indicators in surface waters showed temporal and spatial fluctuations, but statistical analysis did not reveal significant differences among seasons or stations. Numerical variations in heterotrophic bacteria, total coliforms, and fecal coliforms were observed, suggesting potential influence of temperature, organic matter, and agricultural runoff, yet these changes were not statistically significant. Similarly, downstream stations exhibited higher microbial concentrations, but these differences were not statistically significant, suggesting that cumulative upstream pollution and wastewater inputs may contribute without producing consistent statistical effects. Parasitological analysis revealed intermittent Giardia presence, particularly in spring, whereas *Cryptosporidium* was not detected; however, detection limits and the absence of molecular confirmation may affect these findings. These results highlight the necessity of continuous monitoring, especially during spring and summer when numerical increases were noted, while acknowledging that statistical significance was not established. Management actions such as controlling agricultural runoff, minimizing raw wastewater discharge, and enhancing wastewater treatment efficiency remain essential to safeguard water quality.

Author Contributions

Zahra Yaghoubzadeh: Writing-review and editing, methodology, project administration.
Hassan Nasrollahzadeh Saravi: Investigation, methodology.
Sharareh Firouzkandian: Conceptualization.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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Data Availability Statement

The data presented in this study is available on request from the corresponding author.

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