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Assessment of Microbial Indicators of Water Resources in KooheHava and TangeKhoor Free Area

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A B S T R A C T

In the wildlife management, maintaining water quality and quantity, especially in areas that are faced with relative constraints of water resouces, are considered as one of the planning pillars. Natural springs and artificial troughs in the KooheHava and TangeKhoor Free Area are the only sources of water suppliers for wildlife of the area. The aim of this study was to investigate the microbial indices of water resources used by wildlife in this areas and to compare them with the Iranian national standard limit. In this study, 12 water sources including ten springs and two troughs were selected and sampling was carried out in two seasons of summer and autumn of 2020 and three samples from each water resources and a total of 72 samples were collected throughout the study period and the parameters of total coliform, fecal coliform, temperature, turbidity and pH were measured. The data were analyzed by One sample t-test, Paired sample t-test, Independent sample t-test, analysis of variance and Spearman correlation matrix. The results showed that the means of total coliform were higher than the standard limit in all samples with the exception of no. 4 and 12 springs and the means of fecal coliform were higher than the standard limit in all samples with the exception of no. 2, 4, 8, 10, 11 and 12 water resources both during summer and autumn. In summer, with increasing evaporation, the amount of pollution load of water resources was higher than autumn. However in autumn, the number of polluted water resources was higher which was due to the transmission of microbial contaminations caused by human and animal feces via rain. The results of correlation showed a decrease or an increase in turbidity, temperature or pH did not affect the amount of coliforms because despite the strong correlation between total coliform and fecal coliform, no correlation was found between them and physicochemical factors of water. Therefore, considering the temporal and spatial variability of fecal coliforms and their effect on disease, death and reduction of wildlife populations, optimizing and disinfection of water resources with chlorine and dredging them are recommended.

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INTRODUCTION

Currently ecosystems are experiencing the extinction of considerable species that have been largely attributed to human activities. Researches concerning wildlife ecology and biology are vital for improving the way to manage and conserve species against actual biodiversity crisis [1]. Water is an important factor for species and water resources management in habitats plays an important role in conserving wildlife. Lack of access of wildlife to healthy water resources is one of the most important extinction factors of biodiversity and wildlife population decline [2]. Utillization of natural water resources requires knowledge and periodic assessment of their quantity and quality [3, 4] and the best way to manage springs is to support them against any activity led to reduce their quality [5].

It is scientifically impossible and time consuming to search for all pathogenic microbes in water sources. Therefore, water quality is assessed by identifying index microbes which are coliforms (*Enterobacteriaceae* family), common intestinal bacteria. Generally, coliforms are present in a sample of water as a cause of contamination in feces or sewage water in which other case disease microorganisms may be present in the water. Coliforms are of two categories: fecal coliforms are

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present only in the intestine and total coliforms are present in soil and plants in addition to the intestine. Because these index bacteria are highly resistant against inappropriate temperature and pH, if fecal coliform is disappeared due to the unfavorable environment, it can be said surely that no other pathogenic bacteria may survive in that environment. So, measuring coliform in water is a suitable criterion to determine the biological pollution of water, which is the most dangerous type of pollution [6]. The standard limit of number of total coliforms as well as fecal coliforms for drinking water is 0 MPN/100ml [7]. The important genera of coliforms include *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella* and *Escherichia coli* [8].

Generally, domestic animals, wildlife and humans are considered as the main sources of water-borne pathogens [9]. For example, serotype of *Escherichia coli* that inhabit in the intestines of homoeothermic animals cause gastrointestinal infections and acute urinary tract infections [10]. *Escherichia coli* serotypes lead to mastitis, genital and urine tract infection, miscarriage, diarrhea, white feces, and severe dehydration of the body in cattle. *Escherichia coli* are also one of the most common pathogens in poultry. The disease has been seen in chickens, ducks, turkeys and pigeons. In poultry, *Escherichia coli* led to egg duct inflammation and swelling of the pericardium. *Escherichia coli* is also the most common cause of opportunistic urinary tract infection in dogs and cats, and swelling of the urethra and prostate occurs [11]. The *Canidae* (Domestic and wild ones) have become vectors of asymptomatic salmonellosis in many cases and can release *Salmonella* bacteria through feces into the environment [12]. *Klebsiella* can cause pneumonia [13]; *Enterobacter* is somewhat similar to *Escherichia coli* and, like *Klebsiella*, plays some role in urinary tract infections and causes pneumonia, wound infection, and septicemia in hospitalized patients [14]. So, the spread of pathogens in the environment due to human activities may cause many diseases in humans, livestock and wildlife [15]. Having taken into the kinship and genetic affinity of wild species with their domestic counterparts, it can be concluded that those bacteria and pathogens that have been proven to be pathogenic in domestic animals may cause disease and death in wildlife, too.

Today, the consumption of good quality water is one of the vital concerns of living organisms; therefore, continuous monitoring of surficial water quality is necessary [16]. That is why some scientific articles have focused on it in recent years. For example, Pishva [17] in his study concerning the wild Guinea pig fecal pellets in the Karkas Mountain stated that *Escherichia coli* bacteria of human or omnivorous origin polluted water sources and eventually contaminated Guinea pigs with this bacterium. Masoudi et al. [18] in the study of spring water of the northwestern region of Eghlid city concluded that due to the entry of animal and human wastewaters, the mean amounts of coliform in the spring water were higher than the world standard limit and microbial contamination in the springs was higher in July than in March. In the study of river water related to Anzali wetland Faeeid et al. [19] concluded that due to rising ambient temperature and the entry of human wastewater, the amounts of coliform in many stations were higher than the standard limit. In the study of microbial water pollution in the springs of Babol city Aligholizadeh et al. [20] stated that all the samples taken in the two seasons of low rain and high rain were contaminated with coliforms. Haghighat and Nowzari [21] examining a part of Beshar River water quality, indicated that the mean of fecal coliform in the water was higher than the standard limits and the temporal and spatial variability of fecal coliform resulted from the entry of untreated human wastewaters into the river water. In the study of the Sydney watershed, Cox et al. [22] found that the concentrations of pathogens and indicators in the domestic animal's feces was higher than in the wild animal's feces and as a result water contamination from domestic animal's feces was more dominant. Having examined surface water pollution in Nepal, Prasad Ghimire et al. [23] reported that fecal coliform contamination along the sidewalk was due to uncontrolled open waste disposal and poor toilet septic tank conditions. Having studied the surface water of Chalakudy River, Divya and Solomon [24] found that the microbial contamination over standard limit in the water was due to human activities. Having assessed the water sources within the Great Smoky Mountains National Park, Reed and Rasnake [25] stated coliform concentration was higher in the summer than fall in water sources. Having studied the Bailka River, Bojarczuk et al. [26] found that human activities are of the main factors of total coliform and fecal coliform prevalence in the downstream waters of the river. It was concluded that increased upstream human and deer activities caused *Escherichia coli* to exceed the standard limit during the summer months. Having studied the surface water of Usuma River in Nigeria, Ogwueleka and Christopher [27] stated that downstream water quality deterioration of the river indicates the intense spread of human and animal feces and surface runoff.

According to have the same drinking water resources within wildlife and humans (shepherds, rangers, Eco tourists), microbial contamination of these resources may lead to disease spread among mammals, birds and even human communities. Therefore, if the contamination of wildlife drinking water resources is proven, it can be concluded that wildlife populations are strongly affected by water-borne diseases and strategies to prevent pathogenic contamination of water resources should be taken into consideration and urgently create some alternatives to eliminate pathogenic pollution of water sources because it is practically impossible to cure wild animals due to their free movement. Since microbial and bacterial water contamination can have negative effects on health, longevity and size of wildlife populations, the aim of this study was to investigate the microbial indices (total and fecal coliform) of water resources used by wildlife in the KooheHava and TangeKhoor Free Area and to compare them with national standard limits.

MATERIALS AND METHODS

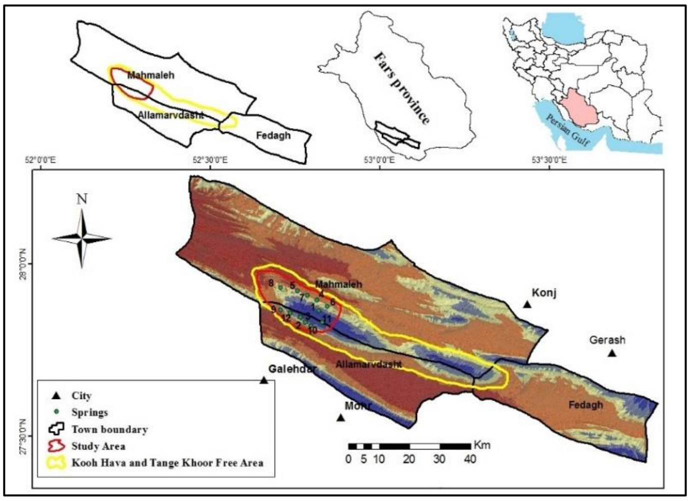
The KooheHava and TangeKhoor Free Area is about 129,658 ha in size and is located from 27˚ 48.6' 22" to 27 ˚56' 36" N and 52˚ 54.25' 44" to 53˚ 21' 66" E. It is located in the southeast of Fars province and by virtue of political divisions, ranges between three districts of Lamerd city (Alamarvdasht district), Khonj (Mahmaleh district) and Gerash (Fadagh district). Its maximum height is 1610 m and is considered as an arid ecosystem. It was established as a no-hunting area in 1999, which is currently conserved as a free area due to its biodiversity reduction. This area includes the mountains of Hava, Shanol, Perpazani and Kuh Talkh, and the confluence of the two ecological zones of the Khalijo-Omani and Irano-Tourani has caused its biodiversity. Its water resources include springs and man-made troughs that are watered by mobile tankers. In addition to exploration and drilling activities for gas extraction by the National Iranian Gas Company, the overgrazing by domestic livestock and various issued mining licenses are among the threats to the area and that is why it has not upgraded enough to be one of the four types of protected area of the Department of Environment [28].

By virtue of the pilot study, having identified its nearest meteorological stations including ones located in Khonj and Lamerd cities, their meteorological data were extracted from the meteorological site of Fars province and by Gaussian method [29], it was determined that August and December were the driest and wettest months in the study area, respectively; that is why sampling was performed during these months. On the other hand, due to the large size and high distribution of the springs and considering the collected samples had to be sent to the laboratory for microbial analysis in the shortest possible time, 12 water sources used mostly by wildlife were selected. Also, field monitoring indicated the largest presence and concentration of wildlife has been around these 12 water sources in the northwestern part of the area (Table 1 and Map 1).

This research was conducted by field works and stratified random sampling. Microbial parameters were collected by sampling from 12 important water sources used by wildlife in the KooheHava and TangeKhoor Free Area during August and December of summer and autumn, 2020. In this way, 3 samples were taken from each water source daily and sent to the laboratory for analysis on the same day. Therefore, 36 samples were collected in each season and the samples were 72 totally. Water sources sampling was performed according to standard water and wastewater conditions in glass containers sterilized with sodium thiosulfate using suitable gloves at a depth of about 20 cm from the water surface [30]. First, the container was filled and emptied once and then the main sample was taken, its temperature, turbidity and pH were extracted and transferred to the laboratory in three hours or less by keeping it cold in a refrigerator and the tests were performed by 9-tube method to count the total coliform in Brilliant Green culture medium by incubator at 35.5 ˚C and the fecal coliform in Ec. Broth culture medium by autoclave (steam bath) at 44.5 ˚C. After passing the standard time for each experiment, the measured parameters were matched with the standard tables according to the

Table 1. Geographical position of the water sources in the study area during 2020

Code	Name	Water source type	Elevation (m)	Latitude (N)	Longitude (E)
1	Narmedoon	Spring	657	27˚ 52′ 58.53″	52˚ 50′ 36.70″
2	Palangi	Spring	489	27˚ 49′ 19.88″	52˚ 46′ 12.78″
3	Parvizi	Spring	501	27˚ 48′ 57.89″	52˚ 47′ 00.81″
4	Kaftari	Spring	554	27˚ 53′ 54.14″	52˚ 49′ 29.68″
5	Mazrae	Spring	661	27˚ 53′ 47.57″	52˚ 48′ 46.09″
6	Galoosorkh	Spring	589	27˚ 51′ 54.35″	52˚ 54′ 09.24″
7	Rasool	Spring	575	27˚ 53′ 39.99″	52˚ 49′ 11.23″
8	Tahran	Spring	623	27˚ 54′ 43.80″	52˚ 47′ 29.66″
9	Abmari	Spring	495	27˚ 51′ 46.60″	52˚ 42′ 22.82″
10	Harar	Trough	1450	27˚ 49′ 44.32″	52˚ 50′ 19.88″
11	Biro	Trough	1380	27˚ 48′ 57.58″	52˚ 51′ 44.02″
12	Mokhi	Spring	483	27˚ 49′ 25.43″	52˚ 46′ 00.28″



Map 1. Geographical position of the study area and the location of sampling stations during 2020

existing scientific instructions and the amounts of total coliform and fecal coliform were announced in MPN/100ml [31].

Normality tests were first used to test data from normality of distribution of frequency. The data were analyzed by One sample t-test to compare the mean of the coliforms with the Iranian national standard limit, Paired sample t-test to compare changes in the coliforms amounts during two seasons, Independent sample t-test and analysis of variance (ANOVA) to compare the parameters of the coliforms in pairs between 12 water sources during two seasons and Spearman correlation matrix to measure the strength of relationships between biological factors such as total coliform and fecal coliform and physicochemical factors such as pH, turbidity and temperature via SPSS software ver.17.0. [32].

RESULTS AND DISCUSSION

According to the result of One sample t-test and the comparison of the mean of total coliform with the Iranian national standard limit, the means of total coliform in all samples except springs no. 4 and 12 had significant difference with the standard limit and were more than the Iranian national standard limit [33] during summer ($0 \leq p \leq 0.05$ for springs no. 1, 2, 3, 5, 6, 7, 8, 9, 10 and 11) (Table 2). Based on the result of One sample t-test and the comparison of the mean of fecal coliform

Table 2. The comparison of the mean ± S.D. of total and fecal coliform amounts of the water sources with the standard limit (0 MPN/100ml) during summer 2020

Water source	Total coliform (MPN/100ml)			Water source	Fecal coliform (MPN/100ml)		
	Mean ± S.D.	*P*-Value	Confidence interval		Mean ± S.D.	*P*-Value	Confidence interval
1	4.60 ± 0.87	0.012	95%	1	2.20 ± 0.00	-	99%
2	5.20 ± 1.65	0.032	95%	2	2.30 ± 1.25	0.086	ns
3	12.40 ± 3.42	0.024	95%	3	5.20 ± 1.65	0.032	95%
4	1.47 ± 0.64	0.057	ns	4	0.00 ± 0.00	-	ns
5	3.60 ± 0.00	-	99%	5	1.83 ± 0.64	0.038	95%
6	9.37 ± 2.55	0.024	95%	6	6.30 ± 1.04	0.009	99%
7	5.70 ± 1.04	0.011	95%	7	3.13 ± 0.81	0.021	95%
8	2.67 ± 0.81	0.029	95%	8	1.47 ± 0.64	0.057	ns
9	8.43 ± 1.33	0.008	99%	9	5.10 ± 0.00	-	99%
10	4.10 ± 0.87	0.015	95%	10	2.30 ± 1.25	0.086	ns
11	2.67 ± 0.81	0.029	95%	11	0.00 ± 0.00	-	ns
12	1.47 ± 0.64	0.057	ns	12	0.37 ± 0.64	0.423	ns

with the Iranian national standard limit, the means of fecal coliform in the springs no. 1, 3, 5, 6, 7 and 9 had significant difference with the standard limit and were more than the Iranian national standard limit [33] during summer (0≤ p ≤0.05 for springs no. 1, 3, 5, 6, 7 and 9) (Table 2).

According to the result of One sample t-test and the comparison of the mean of total coliform with the Iranian national standard limit, the means of total coliform in the all water resources except spring no. 4 had significant difference with the standard limit and were more than the Iranian national standard limit [7] during autumn (0≤ p ≤0.05 for springs no. 1, 2, 3, 5, 6, 7, 8, 9, 10, 11 and 12) (Table 3). Based on the result of One sample t-test and the comparison of the mean of fecal coliform with the Iranian national standard limit, the means of fecal coliform in the all water resources except springs no. 2, 4, 10 and 12 had significant difference with the standard limit and were more than the Iranian national standard limit [7] during autumn (0≤ p ≤0.05 for springs no. 1, 3, 5, 6, 7, 8, 9 and 11) (Table 3).

Analysis of variance (Tukey test) was used to compare the parameters of total coliform and fecal coliform in pairs between 12 water sources during summer and autumn of 2020 and the results of the water sources were significantly different are shown in Tables 4, 5, 6 and 7.

Table 3. The comparison of the mean ± S.D. of total and fecal coliform amounts of the water sources with the standard limit (0 MPN/100ml) during autumn 2020

Water source	Total coliform (MPN/100ml)			Water source	Fecal coliform (MPN/100ml)		
	Mean ± S.D.	*P*-Value	Confidence interval		Mean ± S.D.	*P*-Value	Confidence interval
1	3.13 ± 0.81	0.021	95%	1	1.10 ± 0.00	-	99%
2	3.13 ± 0.81	0.021	95%	2	1.47 ± 0.64	0.057	ns
3	8.43 ± 1.33	0.008	99%	3	3.13 ± 0.81	0.021	95%
4	0.37 ± 0.64	0.423	ns	4	0.00 ± 0.00	-	ns
5	2.20 ± 0.00	-	99%	5	1.10 ± 0.00	-	99%
6	7.07 ± 2.06	0.027	95%	6	1.83 ± 0.64	0.038	95%
7	3.13 ± 0.81	0.021	95%	7	1.10 ± 0.00	-	99%
8	2.20 ± 0.00	-	99%	8	1.10 ± 0.00	-	99%
9	4.10 ± 0.87	0.015	95%	9	1.83 ± 0.64	0.038	95%
10	2.67 ± 0.81	0.029	95%	10	0.73 ± 0.64	0.184	ns
11	1.83 ± 0.64	0.038	95%	11	1.10 ± 0.00	-	99%
12	1.10 ± 0.00	-	99%	12	0.00 ± 0.00	-	ns

Table 4. The comparison of the mean ± S.D. of total coliform amounts in pairs between 12 water sources during summer 2020

W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value
1 3	4.60 ± 0.87 12.40 ± 3.42	0.00**	3 10	12.40 ± 3.42 4.10 ± 0.87	0.00**	6 8	9.37 ± 2.55 2.67 ± 0.81	0.001**
1 6	4.60 ± 0.87 9.37 ± 2.55	0.028*	3 11	12.40 ± 3.42 2.67 ± 0.81	0.00**	6 10	9.37 ± 2.55 4.10 ± 0.87	0.011*
2 3	5.20 ± 1.65 12.40 ± 3.42	0.00**	3 12	12.40 ± 3.42 1.47 ± 0.64	0.00**	6 11	9.37 ± 2.55 2.67 ± 0.81	0.001**
3 4	12.40 ± 3.42 1.47 ± 0.64	0.00**	4 6	1.47 ± 0.64 9.37 ± 2.55	0.00**	6 12	9.37 ± 2.55 1.47 ± 0.64	0.00**
3 5	12.40 ± 3.42 3.60 ± 0.00	0.00**	4 9	1.47 ± 0.64 8.43 ± 1.33	0.00**	8 9	2.67 ± 0.81 8.43 ± 1.33	0.004**
3 7	12.40 ± 3.42 5.70 ± 1.04	0.001**	5 6	3.60 ± 0.00 9.37 ± 2.55	0.004**	9 11	8.43 ± 1.33 2.67 ± 0.81	0.004**
3 8	12.40 ± 3.42 2.67 ± 0.81	0.00**	5 9	3.60 ± 0.00 8.43 ± 1.33	0.025*	9 12	8.43 ± 1.33 1.47 ± 0.64	0.00**

**confidence interval at 99% *confidence interval at 95%

Table 5. The comparison of the mean ± S.D. of fecal coliform amounts in pairs between 12 water sources during summer 2020

W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value
1 3	2.20 ± 0.00 5.20 ± 1.65	0.011*	3 11	5.20 ± 1.65 0.00 ± 0.00	0.00**	6 10	6.30 ± 1.04 2.30 ± 1.25	0.00**
1 6	2.20 ± 0.00 6.30 ± 1.04	0.00**	3 12	5.20 ± 1.65 0.37 ± 0.64	0.00**	6 11	6.30 ±1.04 0.00 ± 0.00	0.00**
1 9	2.20 ± 0.00 5.10 ± 0.00	0.015*	4 1	0.00 ± 0.00 2.20 ± 0.00	0.00**	6 12	6.30 ± 1.04 0.37 ± 0.64	0.00**
2 3	2.30 ± 1.25 5.20 ± 1.65	0.015*	4 7	0.00 ± 0.00 3.13 ± 0.81	0.007**	7 11	3.13 ± 0.81 0.00 ± 0.00	0.007**
2 6	2.30 ± 1.25 6.30 ± 1.04	0.00**	4 9	0.00 ± 0.00 5.10 ± 0.00	0.00**	7 12	3.13 ± 0.81 0.37 ± 0.64	0.023*
2 9	2.30 ± 1.25 5.10 ± 0.00	0.021*	5 6	1.83 ± 0.64 6.30 ± 1.04	0.00**	8 9	1.47 ± 0.64 5.10 ± 0.00	0.021*
3 4	5.20 ± 1.65 0.00 ± 0.00	0.00**	5 9	1.83 ± 0.64 5.10 ± 0.00	0.004**	9 10	5.10 ± 0.00 2.30 ± 1.25	0.021*
3 5	5.20 ± 1.65 1.83 ± 0.64	0.003**	6 7	6.30 ± 1.04 3.13 ± 0.81	0.006**	9 11	5.10 ± 0.00 0.00 ± 0.00	0.00**
3 8	5.20 ± 1.65 1.47 ± 0.64	0.001**	6 8	6.30 ± 1.04 1.47 ± 0.64	0.00**	9 12	5.10 ± 0.00 0.37 ± 0.64	0.00**
3 10	5.20 ± 1.65 2.30 ± 1.25	0.015*						

**confidence interval at 99% *confidence interval at 95%

Table 6. The comparison of the mean ± S.D. of total coliform amounts in pairs between 12 water sources during autumn 2020

W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value
1 3	3.13 ± 0.81 8.43 ± 1.33	0.00**	3 8	8.43 ± 1.33 2.20 ± 0.00	0.00**	5 6	2.20 ± 0.00 7.07 ± 2.06	0.00**
1 4	3.13 ± 0.81 0.37 ± 0.64	0.042*	3 9	8.43 ± 1.33 4.10 ± 0.87	0.00**	6 7	7.07 ± 2.06 3.13 ± 0.81	0.001**
1 6	3.13 ± 0.81 7.07 ± 2.06	0.001**	3 10	8.43 ± 1.33 2.67 ± 0.81	0.00**	6 8	7.07 ± 2.06 2.20 ± 0.00	0.00**
2 3	3.13 ± 0.81 8.43 ± 1.33	0.00**	3 11	8.43 ± 1.33 1.83 ± 0.64	0.00**	6 9	7.07 ± 2.06 4.10 ± 0.87	0.023*
2 4	3.13 ± 0.81 0.37 ± 0.64	0.042*	3 12	8.43 ± 1.33 1.10 ± 0.00	0.00**	6 10	7.07 ± 2.06 2.67 ± 0.81	0.00**
2 6	3.13 ± 0.81 7.07 ± 2.06	0.001**	4 6	0.37 ± 0.64 7.07 ± 2.06	0.00**	6 11	7.07 ± 2.06 1.83 ± 0.64	0.00**
3 4	8.43 ± 1.33 0.37 ± 0.64	0.00**	4 7	0.37 ± 0.64 3.13 ± 0.81	0.042*	6 12	7.07 ± 2.06 1.10 ± 0.00	0.00**
3 5	8.43 ± 1.33 2.20 ± 0.00	0.00**	4 9	0.37 ± 0.64 4.10 ± 0.87	0.002**	9 12	4.10 ± 0.87 1.10 ± 0.00	0.021*
3 7	8.43 ± 1.33 3.13 ± 0.81	0.00**						

**confidence interval at 99% *confidence interval at 95%

Also, the results of Paired sample t-test of changes in fecal coliform amounts during two seasons of summer and autumn of 2020 indicated that the amounts of fecal coliform in the two seasons of summer and autumn of 2020 are significantly different ($p \leq 0.01$) (Table 8).

Table 7. The comparison of the mean ± S.D. of fecal coliform amounts in pairs between 12 water sources during autumn 2020

W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value	W.S	Mean ± S.D.	*P*-Value
1 3	1.10 ± 0.00 3.13 ± 0.81	0.00**	3 6	3.13 ± 0.81 1.83 ± 0.64	0.044*	3 12	3.13 ± 0.81 0.00 ± 0.00	0.00**
2 3	1.47 ± 0.64 3.13 ± 0.81	0.004**	3 7	3.13 ± 0.81 1.10 ± 0.00	0.00**	4 6	0.00 ± 0.00 1.83 ± 0.64	0.001**
2 4	1.47 ± 0.64 0.00 ± 0.00	0.015*	3 8	3.13 ± 0.81 1.10 ± 0.00	0.00**	4 9	0.00 ± 0.00 1.83 ± 0.64	0.001**
2 12	1.47 ± 0.64 0.00 ± 0.00	0.015*	3 9	3.13 ± 0.81 1.83 ± 0.64	0.044*	6 12	1.83 ± 0.64 0.00 ± 0.00	0.001**
3 4	3.13 ± 0.81 0.00 ± 0.00	0.00**	3 10	3.13 ± 0.81 0.73 ± 0.64	0.00**	9 12	1.83 ± 0.64 0.00 ± 0.00	0.001**
3 5	3.13 ± 0.81 1.10 ± 0.00	0.00**	3 11	3.13 ± 0.81 1.10 ± 0.00	0.00**			

**confidence interval at 99% *confidence interval at 95%

Table 8. The results of Paired sample t-test of changes in total and fecal coliform during two seasons of summer and autumn of 2020

Season	Total coliform		Season	Fecal coliform	
	Mean ± S.D.	*P*-Value		Mean ± S.D.	*P*-Value
Summer	5.14 ± 3.50	0.00**	Summer	2.50 ± 2.14	0.00**
Autumn	3.29 ± 2.38		Autumn	1.21 ± 0.89	

**confidence interval at 99% *confidence interval at 95%

The strength of relationships between the parameters of total coliform, fecal coliform, pH, turbidity and temperature were examined by Spearman correlation matrix; the findings indicated a significant positive correlation between total coliform and fecal coliform in summer at 99% confidence interval (Table 9). Also, the findings indicated a significant positive correlation between total coliform and fecal coliform in autumn at 99% confidence interval. On the other hand, there was a significant positive correlation between pH and turbidity, between turbidity and temperature, and between pH and temperature in all three cases at 99% confidence interval (Table 10).

Fecal coliform contamination of water sources is an environmental problem that is of great importance. Identifying the individual host resources of fecal coliform, such as humans, raised and wild animals is a prerequisite for setting up restoration and management programs [34]. In this study, the changes of total and fecal coliform indices were studied in 12 water sources during summer and autumn of 2020 in the KooheHava and TangeKhoor Free Area. The findings of One sample t-test and comparison of the mean of total coliform with

Table 9. The Spearman correlation matrix between measured parameters in the water sources in summer 2020

Parameter	Total coliform	Fecal coliform
Total coliform	-	
Fecal coliform	0.918**	-

**confidence interval at 99% *confidence interval at 95%

Table 10. The Spearman correlation matrix between measured parameters in the water sources in autumn 2020

Parameter	Total coliform	Fecal coliform	pH	Turbidity	Temperature
Total coliform	-				
Fecal coliform	0.772**	-			
pH	0.319	0.194	-		
Turbidity	0.143	0.158	0.456**	-	
Temperature	-0.274	-0.288	0.547**	0.546**	-

**confidence interval at 99% *confidence interval at 95%

the Iranian national standard limit during summer indicated that the means of total coliform in the all water resources except springs no. 4 and 12 (Known as Kaftari and Mokhi) were significantly different from the standard limit and were more than it (Table 2). These findings indicate that springs no. 4 and 12 (Known as Kaftari and Mokhi) were not polluted but other springs were contaminated due to the presence and use of nomads or domestic animals and the passage of rangers, mountaineers or hunters. Also, fewer people or animals have used the Kaftari and Mokhi springs during summer because their paths were difficult to pass. Moreover, the material of the spring bed may play some role in relation to the spring no. 4 (Known as Kaftari) because the bed is stony and due to the gentle slope of the spring the water passes through the rocks seams, gathers in the hollows of the rocks and then overflows; This continuous flow of water prevents the accumulation of microbial contaminants in the Kaftari spring so its water is cleaner. These findings are in accord with the findings reported by Ogwueleka and Christopher's [27]. They found that high levels of total coliforms at various stations were due to human and animal feces. Having compared the mean of fecal coliform with the Iranian national standard limit during summer, the findings indicated that the means of fecal coliform in the all water sources except water sources no. 2, 4, 8, 10, 11 and 12 (Known as Palangi, Kaftari, Tahran, Harar, Biro and Mokhi) were significantly different from the standard limit and were more than the reported source (Table 2) so they were not contaminated because of flowing water and stony bed of the springs no. 2, 4, 8 and 12 (Known as Palangi, Kaftari, Tahran and Mokhi) and artificial water intake of the troughs no. 10 and 11 (Known as Harar and Biro). These findings are in accord with ones of Buckalew et al. [35] study indicating fecal coliform amounts higher than standard limit in the studied stations.

On the other hand, having compared the mean of total coliform with the Iranian national standard limit during autumn, the findings showed that the means of total coliform in the all water sources except spring no. 4 (Known as Kaftari) differed significantly from the standard limit and were more than it (Table 3). In fact, more water sources were infected by total coliforms during autumn compared to summer, however the amounts of total coliform were less in autumn than in summer due to rainfall and increased water in the water sources led to dilution of microbial contamination load. These findings are in accord with Wei et al. [36] and Faeeid et al. [19] studies who found that the means of total coliform exceeded the standard limit. Also, having compared the mean of fecal coliform with the Iranian national standard limit during autumn, the findings showed that the means of fecal coliform in the all water sources except water sources no. 2, 4, 10 and 12 (known as Palangi, Kaftari, Harar and Mokhi) differed significantly from the standard limit and were more than the reported source (Table 3). The findings from the study are in accord with Hackbarth and Weissinger's [37] study who found high concentrations of fecal coliform in water samples collected during summer due to ecotourism and wildlife activity and in accord with Tulagi's [38] study who reported fecal coliform amounts higher than standard limit in the studied stations, too.

As a result, the number of water sources infected by total and fecal coliform during summer was less than autumn, however the amount of pollution load of total and fecal coliform during summer was higher than autumn because of decreasing of spring’s water and water concentration due to lack of rain and lack of flowing water. The spring no. 4 (Known as Kaftari) due to the stony bed and autumn rain that led to reduce the contamination of fecal coliform in the volume of water, the spring no. 2 (Known as Palangi) due to the stony bed and flowing water, the trough no. 10 (Known as Harar) due to artificial watering and plastic walls of it and the spring 12 (Known as Mokhi) due to flowing water, stony bed and difficult access path were free of microbial pollution during both seasons. Generally, excessively standard amounts of fecal coliform in the water of a water source make it risky for human or wildlife consumption in the KooheHava and TangeKhoor Free Area and lead the watery feces to be defecated from wild animals which found too much around springs no. 1, 3, 5 and 6 (Known as Narmedoon, Parvizi, Mazrae and Galoosorkh) during field working. The Reed and Rasnake's [25] findings indicated that the amounts of coliform in water sources were higher in summer than autumn, and the Masoudi et al.'s [18] findings showed that springs were more coliform-polluted in July than March; so their findings are in accord with the present study findings.

The comparison of the means of parameters with independent sample t-test and Tukey's ANOVA indicates almost similar results proving the accuracy of these tests. The findings showed that the means of parameters of total and fecal coliform were significantly different between water sources (Table 4, 5, 6 and 7). During summer the lowest mean values of total and fecal coliform were found in water sources no. 4, 8, 11 and 12 (which did not differ significantly) and the highest mean values of total and fecal coliform were found in springs no. 3, 6 and 9 (which are not significantly different) so that these water sources differed significantly from other ones. These results indicate that springs no. 4 and 12 (Known as Kaftari and Mokhi) due to rock bed and flowing water, spring no. 8 (Known as Tahran) due to flowing water and trough no. 11 (Known as Biro) due to artificial watering and cement-made of walls and bed had the least pollution by total and fecal coliform while spring no. 3, 6 and 9 (Known as Parvizi, Galoosorkh and Abmari) due to the presence of nomads, human and livestock passage and waste disposal were the most contaminated with total and fecal coliform. It is noteworthy that remanents of human life such as worn-out fabrics and clothes, desert toilets, cans, cigarette packs, human waste dumping, livestock cages and manure depots, remains of lost carcasses of domestic animals, disposable tablecloths, beverage bottles, etc. were observed near these microbial-polluted water sources during the field observations. The findings are in accord with Bay et al.'s [39] and Prasad Ghimire et al.'s [23] ones who found that the highest levels of total and fecal coliform contamination were due to human excreta, abnormal open waste disposal and poor toilet disposal conditions and are in accord with Pandey et al.'s [40] and Bojarczuk et al.'s [26] studies who reported that if water ecosystem is used more and more activities were done upstream and around it, the rate of microbial contamination is higher too.

On the other hand, during autumn the lowest mean values of total and fecal coliform were found in water sources no. 4, 10 and 12 (which did not differ significantly) and the highest mean values of total and fecal coliform were found in springs no. 3, 6 and 9 (which are not significantly different) which differed significantly from other ones. These results indicate that springs no. 4 and 12 (Known as Kaftari and Mokhi) due to the stony bed and flowing water and trough 10 (Known as Harar) due to its plastic walls and bed had the least pollution by total and fecal coliform while springs no. 3, 6 and 9 (Known as Parvizi, Galoosorkh and Abmari) due to the presence of nomads, human and livestock passage and feces disposal and transfer of pollution load by rain to these water sources had had the most contamination by total and fecal coliform. The findings of this study are in accord with Zhang et al. [41] ones who stated that rainfall causes more microbial load to enter, Masoudi et al.'s [18] ones who stated that the increase of coliform load and decrease of water quality of springs are because of nomadic habitation and disposal of human and animal wastes, and Haghighat and Nowzari's [21] and Divya and Solomon's [24] studies who stated that human uses increase the total and fecal coliform contamination loads and make water hazardous and pathogenic for wildlife consumption.

The findings of Paired sample t-test related to the changes in total and fecal coliform amounts in autumn compared to summer of 2020 showed that the amounts of total and fecal coliform differed significantly during the two seasons of summer and autumn of 2020 and the amount of total and fecal coliform were lower in all water sources in autumn than in summer (Table 8). Therefore, the main reason for the decrease in total and fecal coliform in autumn compared to summer can be considered because of flooding of water sources and dilution of microbial pollutants due to rainfall. In fact, the rain, on one hand, by washing and leading the surrounding microbial contamination to the water sources, had caused more water sources to be polluted; on the other hand, by spring water increasing, had caused the pollution load to be diluted.

The findings of Spearman correlation matrix indicated that there was a strong significant positive correlation between total coliform and fecal coliform in summer and in autumn namely if a water source had more total coliform, it has more fecal coliform (Table 9). On the other hand, the findings of this test indicated that there was a strong positive correlation between pH and turbidity, between turbidity and temperature, and between pH and temperature in autumn, however there was no correlation between turbidity, pH or temperature with total coliform or fecal coliform; so those factors' increase or decrease did not influence the amounts of coliforms (Table 10).

CONCLUSION

Monitoring the quality of springs through data statistical analysis is one of the most important parts of assessment in the habitat management. The microbial agents of springs vary greatly due to torrential rains, animal invasions, droughts and human interventions. The findings of comparison of water resources microbial indices with the Iranian national standard limits indicated that the means of total coliform were higher than the standard limit in the all water sources except springs no. 4 and 12 (Known as Kaftari and Mokhi) during summer and in the all water sources except spring no. 4 during autumn. It is noteworthy that the stony bed of Kaftari spring plays an important role in its cleanliness, and the paths of Kaftari and Mokhi springs are difficult to pass so human and animal factors are more limited to use them. On the other hand, the means of fecal coliform were higher than the Iranian national standard limit in the water sources no. 1, 3, 5, 6, 7 and 9 (Known as Narmedoon, Parvizi, Mazrae, Galoosorkh, Rasool and Abmari) during summer and in the water sources no. 1, 3, 5, 6, 7, 8, 9 and 11 (Known as Narmedoon, Parvizi, Mazrae, Galoosorkh, Rasool, Tahran, Abmari and Biro) during autumn. Due to the passage of hunters, rangers and the presence of nomads and domestic animals the water of those springs is contaminated after receiving microbial contamination from human, animal or natural sources. Contamination by fecal coliform causes diarrhea and excretion of watery feces, gastrointestinal diseases and blood diseases in animals and eventually their death, and also has a negative effect on the hatching process and infection of newborn chicks so the coliforms have a negative effect on biodiversity and reduce wildlife populations. Therefore, the findings of this study indicated that the number of water sources contaminated by coliforms has increased in autumn compared to summer, however the pollution load amount of coliforms in autumn has been less than in summer. In fact, on one hand, rain has washed away and issued microbial contaminants surrounding the water sources so it has polluted more water sources, and on the other hand, it has reduced the microbial pollution load by diluting the spring's water. Because of human and wildlife consumption from the water sources in the area, the risk of infection, disease and death threaten human and living beings. Temporal and spatial variability of coliforms have been due to the passage of nomads, ecotourists, rangers, poachers, gas company personnel, rainfall and evaporation. Also, the findings of comparing the water sources in terms of microbial indices values indicated during summer, the lowest mean values of total and fecal coliform were found in the water sources no. 4, 8, 11 and 12 due to the stony bed and flowing water of Kaftari, Tahran and Mokhi springs and artificial watering and cement-made of walls and bed of the Biro trough and the highest mean values of total and fecal coliform were found in the springs no. 3, 6 and 9 (Known as Parvizi, Galoosorkh and Abmari) where more nomads lived and more animal and human waste were disposed around them. On the other hand, during autumn, the lowest mean amounts of total and fecal coliform were found in water sources no. 4, 10 and 12 due to the stony bed and flowing water of Kaftari and Mokhi springs and artificial watering and plastic walls and bed of Harar trough and the highest mean amounts of total and fecal coliform were found in the springs no. 3, 6 and 9 due to the reason mentioned above. As a result, in the whole study period, the most microbial-polluted water sources were springs no. 3, 6 and 9 and the cleanest ones were springs no. 4 and 12. The findings indicated that there was a strong positive association between total coliform and fecal coliform in summer and in autumn; that is, each spring had more total coliform it had also more fecal coliform. So, during summer, when evaporation enhances, the microbial contamination load of water sources increases, too whereas during autumn whenever rainfall starts, the springs' water is more diluted then, the microbial pollution load decreases. Generally, dry and wet days and seasons are effective in the microbial indices variations, however in this study, the role of physicochemical factors such as turbidity, temperature and pH was not proven in decreasement or increasement of microbial contamination because despite the strong correlation between those factors, no correlation was found between them and total or fecal coliforms. According to the results of this study, it is recommended to collect and study the animal's feces and their bacterial flora to determine the effect of microbial contamination of water sources on them, enclosure of contaminated water sources to prevent the transmission of microbial agents to wildlife, disinfection and chlorinate the water resources of the area and the tanker's water before discharging them to the troughs, dredging the water sources to purify their water, and control the human pollution occurred at the heights to prevent their transpiration into the water sources especially during rainfall.

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Persian Abstract

چکیده

در مدیریت حیات وحش حفظ کیفیت و کمیت آب به ویژه در مناطقی که با محدودیت نسبی منابع آب مواجه هستند، به عنوان یکی از ارکان برنامه‌ریزی مطرح می‌باشد. چشمه‌های طبیعی و آبشخورهای مصنوعی در منطقه آزاد کوه هوا و تنگ‌خور تنها منابع تأمین‌کننده آب مورد نیاز حیات وحش این منطقه هستند. این پژوهش با هدف بررسی شاخص‌های میکروبی منابع آبی مورد استفاده حیات وحش در این منطقه و مقایسه آنها با استانداردهای ملی صورت گرفت. در این مطالعه ۱۲ منبع آبی شامل ده چشمه و دو آبشخور انتخاب شدند و نمونه‌برداری در دو فصل تابستان و پاییز ۱۳۹۹ انجام شد و از هر منبع آبی سه نمونه و در مجموع ۷۲ نمونه در کل دوره مطالعه جمع‌آوری گردید و پارامترهای کلیفرم کل، کلیفرم مدفوعی، دما، کدورت و pH استخراج گردیدند. داده‌های بدست آمده از طریق آزمون تی تک نمونه‌ای، آزمون تی جفتی، آزمون تی غیرجفتی، آزمون تجزیه واریانس و آزمون همبستگی اسپیرمن مورد تجزیه و تحلیل قرار گرفتند. نتایج نشان داد در تابستان و پاییز میانگین کلیفرم کل در همه منابع آبی به جز چشمه ۴ و ۱۲ و میانگین کلیفرم مدفوعی در همه منابع آبی به جز منابع آبی ۲، ۴، ۸، ۱۰، ۱۱ و ۱۲ از حد مجاز استاندارد ملی ایران فراتر بود. در تابستان با افزایش تبخیر مقدار بار آلودگی منابع آبی بیشتر بود اما در پاییز تعداد منابع آبی آلوده بیشتر بود که به دلیل انتقال آلودگی‌های میکروبی ناشی از فضولات انسانی و حیوانی توسط باران بود. نتایج همبستگی نشان داد کاهش یا افزایش پارامترهای کدورت، دما و pH بر تغییرات مقدار کلیفرم‌ها مؤثر نیست زیرا باوجود همبستگی قوی بین کلیفرم کل و کلیفرم مدفوعی هیچ‌گونه همبستگی بین آنها با فاکتورهای فیزیکوشیمیایی آب بدست نیامد. بنابراین با توجه به تغییرات زمانی و مکانی کلیفرم‌های مدفوعی و تاثیر آنها بر بیماری، مرگ و کاهش جمعیت‌های حیات وحش، بهینه‌سازی و ضدعفونی منابع آب با کلر و لایروبی آنها پیشنهاد می‌گردد.