ORIGINAL ARTICLE

Evaluation of Bacterial Contamination in Sarein Mineral Spas

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Abstract

Background: Failure to observe proper hygiene principles of water and swimming pool environment is effective in causing health problems and transmission of infectious diseases to swimmers. This study aimed to analyze the level of Escherichia coli and Heterotrophic Plate Count in mineral pools in Sarein.

Methods: For this purpose, sampling was performed in each season, and the samples were tested according to the standard method. Shapiro-Wilk and Kolmogorov-Smirnov test was used to determine the normality or abnormality of the data. Then, through ANOVA, the differences between the seasons and the pools were compared in terms of the studied parameters.

Results: The results of the analysis of variance showed that there was no significant difference between the spas in terms of the measured parameters. A comparison of the average data showed that the amount of contamination of mineral spas during the seasons with E. coli was more than the allowable value announced by the National Standard Organization of Iran. The amount of residual chlorine in all samples was zero and the pH was equal to 6.8. The results of principal component analysis showed that mineral spas No. 6, 2, and 11 had the highest HPC (Heterotrophic Plate count) and pH and 9 Cheshmeh, Ershad, and Ghahveh Sui mineral spas based on the second component had the highest E. coli; No. 7 had the lowest E. coli.

Conclusion: Finally, it can be concluded that the cause of the contamination of mineral swimming pools in Sarein city is the high volume of passengers and the lack of proper sanitary management of swimming pools.

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Introduction

Geothermal resources provide a stable warm environment, which is the preferred condition for thermophiles. The composition and properties of hot spring water may vary in terms of organic and mineral chemical elements, pH and nutrients, which may provide favorable conditions for microbial biodiversity.[1-3] Geothermal water is currently used only for recreational purposes in swimming pools.[4] In natural swimming pools, chemical disinfection replaces natural biological processes for water treatment.[5] Despite the growing popularity of these natural systems, little is known about the effectiveness of natural processes in eliminating intestinal pathogens in swimmers. It is also well known by health officials that most recreational illnesses are not recognized in regular health care programs,[6] so the majority are not reported.[7] In general, the chlorine-resistant parasitic protozoan, Cryptosporidium hominis, is the leading cause of gastroenteritis in swimming pools which is caused by fecal diffusion.[8-12]

Human feces may contain pathogens[13-16] and is a known route of infection in recreational aquatic environments.[17-20] Hence, in a natural pool with no

chemical disinfectant residues, pathogens persist until the pool water containing human intestinal viruses, which are the most numerous and most infectious of these intestinal pathogens, cross sufficient natural barriers.[21-24] It should be noted that many pollutants such as other microorganisms and nutrient compounds can be present in aquatic environments naturally or due to human activity.[25-31] Also, mineral and natural compounds or different toxic compounds can be found in the hot water mediums.[32-37]

Sarein city in Ardabil province is very popular due to the existence of numerous geothermal hot springs with a temperature of 45 degrees Celsius. These springs are heated by an inactive volcano in the second highest mountain of Iran, Sabalan.[38] Several researchers have studied the biological contamination of Sarein hot mineral water pools. The study of Sadeghi et al.[39] which aimed to evaluate the quality of mineral spas in Sarein tourist city in Ardabil province showed that, in general, during 5 months of sampling of mineral spas, only 18.82% of the samples were in the standard range compared to the allowable limit of Staphylococcus aureus. Therefore, 81.18% pollution in mineral spas of Sarein city has a risk of skin diseases and possible infections for swimmers. Feyzolahpour et al.[40] observed that due to complete disinfection of pools with chlorine, no dermatophytes were found in Sarein mineral spa. In the study of Seyedmousavi et al.,[41] Aspergillus fumigatus with 22.79%, Aspergillus feldous with 15.54%, Aspergillus niger with 15.54%, and Penicillium with 14.5% had the highest frequency of isolated fungi, respectively. Also, each of the fungi of Ulocladium, Sepdonium, Acromonium, Psilomasis, Stemphylium and Streptomyces with a positive plate of 0.51% had the lowest frequency. In their study, no dermatophyte fungi were isolated from carpet and water samples. Also, no real dimorphic pathogen was isolated in their study. The absence of dermatophyte pathogenic fungi and fungi causing superficial and cutaneous fungal diseases showed that training workers, continuous washing and disinfection of swimming pools, and observance of hygienic standards have been effective in reducing pollution; on the other hand, hot mineral water can prevent the growth of pathogenic fungi. Therefore, it is necessary to conduct various studies on the biological contamination of hot mineral water pools at specific intervals to prevent the occurrence of health consequences due to biological contamination of these pools. Therefore, in this study, the biological pollution of mineral spas in Sarein city was investigated.

Methods

This is a descriptive-analytical study that was conducted during 2019 to determine the microbial quality in the water of Sarein mineral pools. In this study, samples were randomly taken from 13 pools in Sarein, including mineral pools with codes 1 to 13.

Steps of Microbial Water Testing

1. Possible stage: In this stage, we use broth lactose culture medium with two weak and strong dilutions, so that we put three tubes of strong lactose broth and six tubes of weak lactose broth in one tube, respectively. In the first three tubes where lactose broth is strong, we add 10cc of water; in the second three tubes where lactose broth is weak, we add 1cc, and in the third three tubes, which are also weak lactose broth, we add 0.1cc of the sample. Then, we add water. The tubes are then shaken and placed in an incubator at 35.5-37 degrees for 24-48 hours. At this stage, the possibility of bacteria is checked and reported with the MPN unit per 100 ml.

2. Confirmation stage: In this stage, the culture medium of brilliant green and EC broth is used. Thus, from the positive samples of the first stage (carbonated tubes), we transfer the lactose broth culture medium to these two media. We place the diamond tube inside the incubator and the EC broth inside the 44.5-degree pan. We review the results after 24 hours. If both tubes are negative, it means that there is no problem with water, and the first stage that was positive was bacteria other than coliforms, and the water is drinkable; yet, if the brilliant tube is positive and the EC tube is negative, the water has coliforms and the amount of MPN is reported. It is possible, but, in terms of fecal form, (E. coli) if it is negative and the water is chlorinated, it is drinkable. If both diamond and EC pipes are positive, as well as the coliform, the water also has E.coli and is not drinkable.

Analysis

This is an analytical-descriptive study. The statistical population included 13 active swimming pools in Sarein city, including codes 1 to 13, which were examined for 1 year in each season. In this study, sampling was performed from each pool in the required number each month according to the standard conditions and was transferred to the reference laboratory of Ardabil University of Medical Sciences for analysis. E. coli bacteria[10] were measured in the laboratory. The diagnosis method of E. coli was 15 tubes and the use of lactose broth and EC media.

The results of microbial tests were recorded in Excel, and SPSS software[42, 43] was used for statistical analysis of data. MINITAB15 software was used to draw the cluster analysis and principal components analysis. The Shapiro Wilk and Kolmogorov-Smirnov tests were used to determine whether the data were normal or abnormal. Then, through ANOVA,[44] the differences between the seasons and the pools were compared in terms of the studied parameters. T-test

was used to compare the data with the national standard of Iran.

Results

Before performing statistical analyses, Shapiro-Wilk and Klemgorov-Smirov (K-S) tests were performed to evaluate the normality of data distribution in the studied variables. The results showed that the studied variables had a normal distribution (sig> 0.05). Therefore, the results of analysis of variance, T-test, cluster analysis and PCA were interpreted and analyzed using statistical methods of data analysis.

Analysis of Variance and Comparison of Mean Data

For the data obtained from the measurement of the studied parameters in 13 spa pools of Sarein city, analysis of variance was performed by considering monthly sampling periods as replication and types of spas as treatment using SPSS software. The results of analysis of variance (Table 1) for pH, HPC (Heterotrophic Plate count), and E. coli parameters that were measured in 13 spas and in different time periods showed that there was no significant difference between the spas in terms of the parameters measured. However, to ensure the evaluation of the water quality of the spas in terms of the parameters measured and to complete the information about each parameter separately for the spas, the average with the data was checked.

The results showed that there was no significant difference in pH between mineral water samples. Samples No. 7, 4, and 10 had the highest amount of E. coli and sample No. 13 had the lowest amount of E. coli. Also, in terms of HPC, the highest amount was related to waters No. 11, 2, 6 and 1 and the lowest to number 13.

T-test Statistical Test Results

T-test was performed to know the microbial status of mineral spas in Sarein by comparing the measured values of E. coli and HPC parameters with the national standard values of Iran for hot mineral waters (Table 2). The values of Iran national standards for hot mineral waters are presented in Table 2.

High levels of E. coli in pools No. 1, 2, 4, 6, 7, 8, and 11 (Royal Park, 9 Cheshmeh, Iranian, Ghahveh Sui, General, Sabalan, Ershad) in different seasons of the year were more than the allowance of the National Standard Organization of Iran due to the lack of using showers by swimmers at the entrance or exit of these spas in Sarein. Also, the levels of HPC in pools 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 in different seasons of the year were more than the allowable limit of the National Standard Organization of Iran due to lack of timely water change of swimming pools and not washing the walls and floors of swimming pools in Sarein city.

Cluster Analysis

Cluster analysis is one of the multivariate analysis methods that aims to group the studied spas based on their qualitative characteristics so that spas with similar quality characteristics are in a group. According to the predetermined criteria, grouping will be appropriate when the stations within the groups have more homogeneity and are more heterogeneous between the groups. Investigating the relationship between 3 parameters in 13 spas for the measured parameters requires the analysis of 13×3 correlation matrix. On the other hand, it is necessary to check the condition of the spas and the amount of their pollution by considering all the parameters. Therefore, due to the large volume of data, clustering parameters and spas were performed using cluster analysis based on dissimilarity matrices. Minitab22 software was used for this purpose. To perform cluster analysis, we used the input method based on the minimum square of Euclidean distance after standardizing the data. The average sampling periods for each parameter were used to classify the measured quality parameters in the studied spas of Sarein city and determine their microbial contamination status. Dendrogram section based on the farthest Euclidean distance divided hot waters into four groups. Figure 1 shows the dendrogram of Sarein spas clusters analysis based on the measured parameters.

The first group (first cluster) included No. 1 Royal Park Spa. This spa pool was higher than the average of the measured values in terms of pH and HPC parameterS (Table 3), and the quality changes

Table 1: Mean squares obtained from analysis of variance of measured parameters in hot springs of Sarein city in 2019

Sources of change	pH			E. coli			HPC (Heterotrophic Plate count)		
	Sig.	F	MS	Sig.	F	MS	Sig.	F	MS
Stations (intergroup)	0.34	1.9	207181.2	0.133	1.473	0.004	0.753	0.968	921.679
Error (within group)	-	-	109025.4	-	-	0.003	-	-	1320.638

**, * and ns: Show significance at 1% probability level, significance at 5% probability level and non-significance, respectively.

Table 2: National Standards of Iranfor mineral pools

Type of microorganism	Allowable number of bacteria
Heterotrophic bacteria	200 per milliliter
E. coli or thermophilic coliforms	Less than 2 per 100 ml

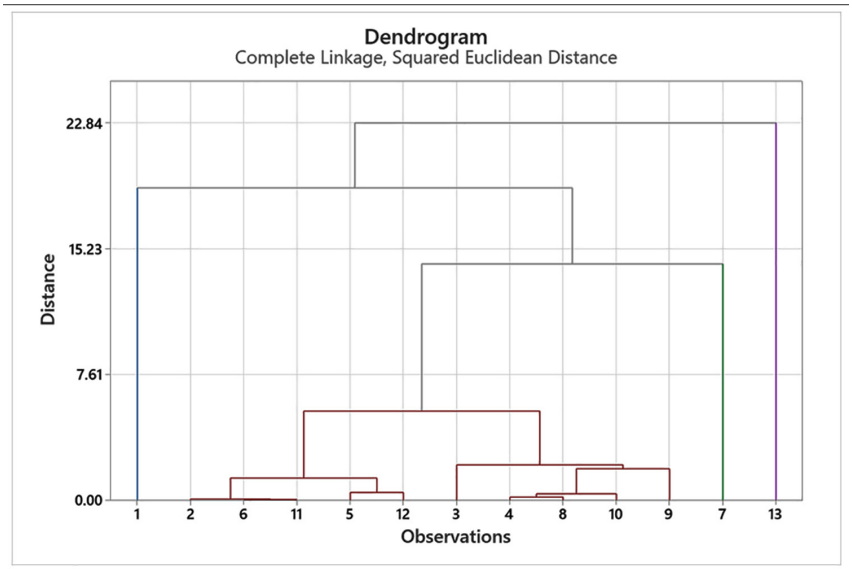

Figure 1: Dendrogram obtained from the cluster analysis of the studied spa based on PC1 (Principle Component) and PC2 (Principle Component) values for the parameters measured in Sarein

Table 3: Mean deviation from total mean and standard deviation of means in 3 clusters obtained from cluster analysis for the evaluated parameters

Cluster	E. coli	pH.	Statistical parameter
1	7.2414	6.8414	$\bar{x}$
2	5.5498	6.8010	$\bar{x}$
3	23.1739	6.8	$\bar{x}$
4	0.00	6.8	$\bar{x}$
Average	6.6087	6.804	Total

* and **: Show significance between clusters at 5% and 1% probability levels, respectively.

of this spa were mainly due to the high volume of customer reception and lack of timely change of pool water and washing of the pool area. The second group (second cluster) including the spas No. 10, 8, 4, 3, 12, 5, 11, 6, 2, and 9 were ranked second in terms of HPC parameter. E. coli and pH were also lower. Changes in these spas are mainly due to non-compliance with health protocols, failure to change the water of swimming pools in a timely manner, and the swimmers' failure to shower before entering the swimming pools. The quality of 9 Cheshmeh, Ghahveh Sui, and Ershad spas is due to the provision of part of the pool water by Pahnloo spring, high number of the swimmer's visit to the swimming pool, non-timely change of swimming pool water, washing and disinfection of the pool area and the swimmers' shower before entering the swimming pool; also, the quality of spas No. 5 and 12 (Darreh Lor Sui and Bash Bajilar) is due to the provision of part of the swimming pool water by Pahnloo spring, the high number of the swimmer's visit to the swimming pool, failure to changing the swimming pool water in time, washing and disinfection of the pool area, the swimmers' shower before entering the pool. Moreover, the quality of Sari Sui spa is due to not changing the water of the swimming pools in time, washing and disinfecting the pool area, the swimmers' shower before entering the pool; also, the quality of Sabalan spa is due to providing part of the pool water from Gavmish Goli, the high number of the swimmers visiting the swimming pool, not changing the water of the swimming pool in time, washing and disinfecting the inside of the pool and swimmers' shower before entering the pool. The quality of the Iranian spa is due to the supply of part of the pool water from Gavmish Goli, high number of the swimmer's visit to the swimming pool, lack of timely change of swimming pool water, washing and disinfection of the pool area and swimmers' shower before entering the pool and the quality of Aftab city spa is due to providing part of the pool water from Pahnloo spring, high volume of swimmer visits to the swimming pool, failure to change the swimming pool water in time, washing and disinfection of the pool area and swimmers' shower before entering the pool; finally, the quality of Gavmish Goli spa is due to the high number of swimmers visiting the swimming pool, not changing the water of the swimming pools in time, washing and disinfecting the pool area and swimmers' shower before entering the pool. As to the third group (third cluster) in which General spa was placed alone in the group, its quality is almost average, which is due to the timely change of water in swimming pools, washing and disinfection of the interior part of the pool and the swimmers' shower before entering the pool. As to the fourth group (fourth cluster) in which

Viladragh spa was placed alone, its quality is desirable due to the low volume of the swimmer's visit to the swimming pool, timely change of swimming pool water, washing and disinfection of the pool area and taking a shower before entering the pool. Therefore, in general, it can be stated that Royal Park Mineral Spa No. 1 has a higher pH and HPC. The General Spa No. 7 has the lowest amount of E. coli, and Viladragh Spa No. 13 is desirable in terms of all parameters. After that, the second group with Moderate pollution (MP) included Gavmish Goli, Sabalan, Iranian, Shahr Aftab, Bash Bajilar, Darreh Lor Sui, Ershad, Ghahveh Sui, 9 Cheshmeh, and Sari Sui spas; the rest of the stations are in the Low pollution group (LP). The results of one-way analysis of variance confirmed the existence of significant differences between the groups in terms of most of the studied parameters at the probability level of 1% and 5%. Examination of the differences between the groups showed that the spas within each cluster did not differ significantly in terms of the measured parameters. However, there was a significant difference between the clusters in terms of most of the evaluated characteristics at the probability levels of 1% and 5% (Table 3).

Results of PCA

The results of PCA (Principle Component Analysis) showed that of the three main components, the first two components explained more than 71% of the changes, so that in the formation of the first component, HPC, and pH had the highest positive effect and in the second component, E. coli had the most positive effect. Therefore, mineral spas No. 6, 2, and 11 (Ghahveh Sui, 9 Cheshmeh and Ershad) had the highest HPC and pH and 9 Cheshmeh, Ershad and Ghahveh Sui mineral spas, based on the second component, had the highest E. coli; also, General mineral spa No. 7 had the lowest E. coli.

The eigenvectors and eigenvalues of the parameters in the PCA (Principle Component Analysis) are presented in Tables 4 and 5, respectively.

Discussion

Based on the results of this study, there was no significant difference between the spas in terms of the measured pH and E. coli parameters. From a public health perspective, frequent measurement of disinfectant concentrations and pH is a very effective way to control water quality.[45] The pH of mineral waters does not differ significantly. However, in the study of Yazdanbakhsh et al.,[46] different pools had different pH levels, which was statistically significant. The water No. 7, 4, and 10 had the highest amount of E. coli, and the water with code 13 had the lowest amount of E. coli. As to the mean data of E. coli parameter in spa pools No. 2 and 12 (9 Cheshmeh and Bash Bajilar) with the national standard of Iran for mineral spa pools, there was a significant difference in terms of E. coli at 99% probability level. High levels of E. coli in swimming pools No. 1, 2, 4, 6, 7, 8, and 11 (Royal Park, 9 Cheshmeh, Iranian, Ghahveh Sui, General, Sabalan, Ershad) in different seasons of the year were more than the allowed limit by the National Standard Organization of Iran, due to the lack of use of showers by swimmers at the entrance or exit of these spas in Sarein. In a study by Masoud et al.,[47] the presence of E. coli in pool water showed fecal matter from contaminated skin or fecal matter which entered the pool water accidentally. It was also found that the treatment process could not remove this contamination. In their study, E. coli was the most consistent index and was isolated from 7 out of 120 samples. Similar results have been reported by Leoni et al.[48] In their study, only 2.6% of the samples showed positive results. Abd El-Salam[49] also reported that E. coli was not found despite the low concentration of chlorine in most samples. This may be attributed to the relatively small sample size because only 30 water samples were examined in their study.

Conclusion

In this study, multivariate statistical methods of analysis of variance, T-test, cluster analysis, and PCA (Principle Component analysis) were used to determine the quality of spas in the tourist city of Sarein in terms of microbial parameters of E. coli, HPC, and alkalinity. The two-dimensional display of the stations based on the first two main components confirmed the grouping of the results obtained from the cluster analysis as well as the principal components analysis; thus, the studied spas were separated in terms of microbial contamination status as cluster analysis. Using cluster analysis method, we categorized 13 sampling stations into four clusters with

Table 4: The eigenvectors of parameters

Variable	PC1 (Principle Component)	PC2 (Principle Component)	PC3 (Principle Component)
pH	0.595	-0.552	-0.584
E. coli	-0.293	-0.826	0.482
HPC	0.749	0.115	0.653

Table 5: The eigenvalues of parameters

Eigenvalue	1.1189	1.0388	0.8423
Proportion	0.373	0.346	0.281
Cumulative	0.373	0.719	1.000

similar quality characteristics. In order to determine the effective parameters in microbial contamination of spas in the tourist city of Sarein, we performed PCA (Principle Component analysis) in four different qualitative groups. The first two main components of PCA (Principle Component analysis) revealed that the major parameters responsible for changes in the microbial quality of spas in Sarein were E. coli and HPC. Also, the amount of HPC and E. coli bacteria in the swimming pools of Sarein city in different seasons of the year were higher than the national standard of Iran.

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Authors' Contribution

Conceptualization: Behnam Afsar. Data curating: Behnam Afsar. Formal analysis: Ali Akbar Imani. Investigation: Behnam Afsar. Methodology: Ebrahim Fataei. Project administration: Ebrahim Fataei. Resources: Behnam Afsar. Software: Ali Akbar Imani. Supervision Ebrahim Fataei. Validation: Ali Akbar Imani. Visualization: Ebrahim Fataei. Writing–original draft: Behnam Afsar. Writing–review & editing: Ebrahim Fataei

Ethical Approval

There was no need for ethical considerations in conducting this study.

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Conflict of Interest: None declared.

References

1 Khalil A. Screening and characterization of thermophilic bacteria (lipase, cellulase and amylase producers) from hot springs in Saudi Arabia. Journal of Food, Agriculture and Environment. 2011;9(2):672-5. doi: 10.1234/4.2011.2187.

2 Shajahan S, Ganesh Moorthy I, Sivakumar N, Selvakumar G. Statistical modeling and optimization of cellulase production by Bacillus licheniformis NCIM 5556 isolated from the hot spring, Maharashtra, India. Journal of King Saud University-Science. 2017;29(3):302-10. doi:10.1016/j.jksus.2016.08.001.

3 Valiallahi J, Moradi S. Evaluating the Effects of Agricultural Activities on Nitrate Contamination at the Kamfirooz District, Shiraz, Iran. Anthropogenic Pollution. 2020;4(1):24-35. doi: 10.22034/AP.2020.1882082.1055.

4 Kaczmarczyk M, Tomaszewska B, Operacz A. Sustainable utilization of low enthalpy geothermal resources to electricity generation through a cascade system. Energies. 2020;13(10):2495. doi: 10.3390/en13102495.

5 Petterson S, Li Q, Ashbolt N. Screening Level Risk Assessment (SLRA) of human health risks from faecal pathogens associated with a Natural Swimming Pond (NSP). Water Research. 2021;188:116501. doi: 10.1016/j.watres.2020.116501. PMID: 33091804. PMCID: PMC7535628.

6 Fewtrell L, Kay D. Recreational water and infection: a review of recent findings. Current environmental health reports. 2015;2:85-94. doi: 10.1007/s40572-014-0036-6. PMID: 25821715. PMCID: PMC4371824.

7 Dwight RH, Fernandez LM, Baker DB, Semenza JC, Olson BH. Estimating the economic burden from illnesses associated with recreational coastal water pollution—a case study in Orange County, California. Journal of environmental management. 2005;76(2):95-103. doi: 10.1016/j.jenvman.2004.11.017.

8 Ghanavat Amani M, Jalilzadeh Yengejeh R. Comparison of Escherichia coli and Klebsiella Removal Efficiency in Aquatic Environments Using Silver and Copper Nanoparticles. Journal of Health Sciences & Surveillance System. 2021;9(2):72-80. doi: 10.30476/jhsss.2021.88646.1151.

9 Kazemi Noredinvand B, Takdastan A, Jalilzadeh Yengejeh R. Removal of organic matter from drinking water by single and dual media filtration: a comparative pilot study. Desalination and water treatment. 2016;57(44):20792-9. doi: 10.1080/19443994.2015.1110718.

10 Mohammadi Aloucheh R, Baris O, Asadi A, Gholam Zadeh S, Kharat Sadeghi M. Characterization of Aquatic Beetles Shells (Hydraenidae family) derived chitosan and its application in order to eliminate the environmental pollutant bacterial. Anthropogenic Pollution. 2019;3(2):43-8. doi: 10.22034/ap.2019.668483.

11 Suppes LM, Canales RA, Gerba CP, Reynolds KA. Cryptosporidium risk from swimming pool exposures. Int J Hyg Environ Health. 2016;219(8):915-9. doi: 10.1016/j.ijheh.2016.07.001. PMID: 27432615.

12 Yousefi M, Rahmani K, Jalilzadeh Yengejeh R, Sabzalipour S, Goudarzi G. Green Synthesis of Zero Iron Nanoparticles and its Application in the Degradation of Metronidazole. Journal of Health Sciences & Surveillance System. 2021;9(1):66-70. doi: 10.30476/jhsss.2020.88584.1146.

13 Fathi H, Mirabbasi SB, Pournoori M. Investigating the

place of international law in the protection of the marine environment against Transportation of hazardous chemicals. Anthropogenic Pollution. 2022;6(1):55-65. doi: 10.22034/AP.2022.1942517.1120.

14 Jalalzadeh A, Rabieifar HR, Vosoughifar H, Razmkhah A, Fataei E. Investigation of daily waste load allocation in Zarrineh-rud river for environmental management of cold-water fish species. Anthropogenic Pollution. 2022;6(1):36-46. doi: 10.22034/ap.2022.1941381.1118.

15 Mahdi A, Karimi D, Farshchi P, Panahi M. Waste Water Pollution zoning of sensitive coastal-marine areas with an environmental protection approach (Study area: Boujagh International Park and International Wetland). Anthropogenic Pollution. 2021;5(1):16-30. doi: 10.22034/ap.2021.1910782.1080.

16 Rasool FN, Saavedra MA, Pamba S, Perold V, Mmochi AJ, Maalim M, et al. Isolation and characterization of human pathogenic multidrug resistant bacteria associated with plastic litter collected in Zanzibar. J Hazard Mater. 2021;405:124591. doi: 10.1016/j.jhazmat.2020.124591. PMID: 33301968.

17 Chalmers RM. Waterborne outbreaks of cryptosporidiosis. Annali dell'Istituto superiore di sanita. 2012;48:429-46. PMID: 23247139. doi: 10.4415/ANN_12_04_10.

18 Dale K, Kirk M, Sinclair M, Hall R, Leder K. Reported waterborne outbreaks of gastrointestinal disease in Australia are predominantly associated with recreational exposure. Aust N Z J Public Health. 2010;34(5):527-30. doi: 10.1111/j.1753-6405.2010.00602.x. PMID: 21040184.

19 Graciaa DS, Cope JR, Roberts VA, Cikesh BL, Kahler AM, Vigar M, et al. Outbreaks Associated Untreated with Recreational Water - United States, 2000-2014. MMWR Morb Mortal Wkly Rep. 2018;67(25):701-6. doi: 10.15585/mmwr.mm6725a1. PMID: 29953425. PMCID: PMC6023190.

20 Pond K. Water recreation and disease: plausibility of associated infections: acute effects, sequelae, and mortality. 2013. doi: 10.2166/9781780405827.

21 Abbasi A, Taghavi L, Sarai Tabrizi M. Qualitative Zoning of Groundwater to Assessment Suitable Drinking Water Using GIS Software in Mohammad Shahr, Meshkinshahr, and Mahdasht in Alborz Province. Anthropogenic Pollution. 2021;5(1):138-49. doi: 10.22034/AP.2021.1907787.1076.

22 Ashbolt NJ. Microbial contamination of drinking water and human health from community water systems. Current environmental health reports. 2015;2:95-106. doi: 10.1007/s40572-014-0037-5. PMID: 25821716.

23 Babaei AA, Ghanbari F, Yengejeh RJ. Simultaneous use of iron and copper anodes in photoelectro-Fenton process: concurrent removals of dye and cadmium. Water science and technology. 2017;75(7):1732-42. doi: 10.2166/wst.2017.049. PMID: 28402315.

24 Jalili S. Water Quality Assessment Based on HFB I& BMWP Index In Karoon River, Khouzestan Provience,(Northwest of Persian Gulf). Anthropogenic Pollution. 2020;4(1):36-49. doi: 10.22034/AP.2020.1877482.1047.

25 Abbaspour M, Javid AH, Jalilzadeh Yengjeh R, Hassani AH, Mostafavi PG. The biodegradation of methyl tert-butyl ether (MTBE) by indigenous Bacillus cereus strain RJ1 isolated from soil. Petroleum Science and Technology. 2013; 31(18):1835-41. doi: 10.1080/10916466.2011.611562.

26 Gashtasbi F, Yengejeh RJ, Babaei AA. Adsorption of vancomycin antibiotic from aqueous solution using an activated carbon impregnated magnetite composite. Desalination and water treatment. 2017;88:286-97. doi: 10.5004/dwt.2017.21455.

27 Jalilzadeh Yengejeh R, Pourjafarian V, Afrous A, Gholami A, Maktabi P, Sharifi R. Studying Bacillus cereus's ability to biodegrade crude oil in hot areas. Petroleum Science and Technology. 2017;35(3):287-91. doi: 10.1080/10916466.2014.941068.

28 Jalilzadeh Yengejeh R, Sekhavatjou MS, Maktabi P, ARBAB SN, Khadivi S, Pourjafarian V. The Biodegradation of Crude Oil by Bacillus subtilis Isolated from Contaminated Soil in Hot Weather Areas. International Journal of Environmental Research. 2014;8(2):509-14. doi: 10.22059/ijer.2014.744.

29 Kordestani B, Takdastan A, Yengejeh RJ, Neisi AK. Photo-Fenton oxidative of pharmaceutical wastewater containing meropenem and ceftriaxone antibiotics: influential factors, feasibility, and biodegradability studies. Toxin reviews. 2018. doi: 10.1080/15569543.2018.1520261.

30 Shokri R, Yengejeh RJ, Babaei AA, Derikvand E, Almasi A.. UV activation of hydrogen peroxide for removal of azithromycin antibiotic from aqueous solution: determination of optimum conditions by response surface methodology. Toxin Reviews. 2020;39(3):284-91. doi: 10.1080/15569543.2018.1517803.

31 Zangeneh A, Sabzalipour S, Takdatsan A, Jalilzadeh Yengejeh R, Abullatif Khafaie M. Ammonia removal form municipal wastewater by air stripping process: An experimental study. South African Journal of Chemical Engineering. 2021;36:134-41. doi: 10.1016/j.sajce.2021.03.001.

32 Aharia SM, Yangejehb RJ, Mahvic AH, Shahamatd YD, Takdastane A. A new method for the removal of ammonium from drinking water using hybrid method of modified zeolites/catalytic ozonation. Desalin Water Treat. 2019;170:148-57. doi: 10.5004/dwt.2019.24619.

33 Farsani MH, Yengejeh RJ, Mirzahosseini AH, Monavari M, Hassani AH, Mengelizadeh N. Effective leachate treatment by a pilot-scale submerged electro-membrane bioreactor. Environ Sci Pollut Res Int. 2022;29(6):9218-31. doi: 10.1007/s11356-021-16196-0. PMID: 34494194.

34 Mehrdoost A, Jalilzadeh Yengejeh R, Mohammadi MK, Babaei AA, Haghighatzadeh A. Comparative Analysis of UV-assisted Removal of Azithromycin and Cefixime from Aqueous Solution Using PAC/Fe/Si/Zn Nanocomposite. Journal of Health Sciences &

Surveillance System. 2021;9(1):39-49. doi: 10.30476/jhsss.2020.88564.1149.

35 Mehrdoost A, Yengejeh RJ, Mohammadi MK, Haghighatzadeh A, Babaei AA. Adsorption removal and photocatalytic degradation of azithromycin from aqueous solution using PAC/Fe/Ag/Zn nanocomposite. Environ Sci Pollut Res Int. 2022;29(22):33514-27. doi: 10.1007/s11356-021-18158-y. PMID: 35029828.

36 Nikpour B, Jalilzadeh Yengejeh R, Takdastan A, Hassani AH, Zazouli MA. The investigation of biological removal of nitrogen and phosphorous from domestic wastewater by inserting anaerobic/anoxic holding tank in the return sludge line of MLE-OSA modified system. J Environ Health Sci Eng. 2020;18(1):1-10. doi: 10.1007/s40201-019-00419-1. PMID: 32399216. PMCID: PMC7203377.

37 Hussain S, Aslam MU, Javed M, Zahra M, Ejaz H, Kubra KA, Mushtaq I. Impact of Climatic Changes and Global Warming on Water Availability. Anthropogenic Pollution. 2021;5(2):57-66. doi: 10.22034/ap.2021.1926893.1100.

38 Salehi S, Yari M, Rosen MA. Exergoeconomic comparison of solar-assisted absorption heat pumps, solar heaters and gas boiler systems for district heating in Sarein Town, Iran. Applied Thermal Engineering. 2019;153:409-25. doi: 10.1016/j.applthermaleng.2019.03.022.

39 Sadeghi H, Bagheri Ardebilian P, Rostami R, Poureshgh Y, Fazlzadeh M. Biological and physicochemical quality of thermal spring pools, with emphasis on Staphylococcus aureus: Sarein tourist town, Ardabil. Journal of Environmental Health Enginering. 2014;1(3):203-15.

40 Feyzolahpour M, Einali J, Gasemlu H. Investigating the geotouristic risks of spa springs with emphasis on natural and human dangers in the study area between Sarein and Ardebil in the northwest of Iran. AUC GEOGRAPHICA. 2019;54(2):194-206. doi: 10.14712/23361980.2019.17.

41 Seyedmousavi S, Fataei E, Hashemi S, Geramishoare M. Fungal Flora in Mineral Swimming Pools of Sarein-Iran (2005). J Ardabil Univ Med Sci. 2007;7(2):146-54.

42 Farhadi H, Fataei E, Kharrat Sadeghi M. Kharrat Sadeghi, The Relationship Between Nitrate Distribution in Groundwater and Agricultural Landuse (Case study: Ardabil Plain, Iran). Anthropogenic Pollution Journal. 2020;4(1):50-6. doi: 10.22034/AP.2020.1885788.1059.

43 Zaeimdar M, Tavakoli F, Lahijanian A. The Impact of Environmental Education on The Environmental Behavior of Elementary School Students (Case Study: Students in Tehran). Anthropogenic Pollution. 2019;3(2):37-42. doi: 10.22034/AP.2019.668482.

44 Ramazani ME, Mosaferi M, Abedi S. Development of Management Strategies for the Urban Environment with SWOT Approach: A Case Study in Iran. Anthropogenic Pollution. 2017;1(1):25-33. doi: 10.22034/APJ.2017.1.1.2533.

45 Vukić Lušić D, Maestro N, Cenov A, Lušić D, Smolčić K, Tolić S, et al. Occurrence of P. aeruginosa in Water Intended for Human Consumption and in Swimming Pool Water. Environments. 2021;8(12):132. doi: 10.3390/environments8120132.

46 Yazdanbakhsh AR, Mohseni bandpei A, Bay, A Sadeghi. The relationship between physicochemical and microbial indicators in Jacuzzi water and swimming pools in Golestan Province. Journal of Research in Environmental Health. 2016;2(1):71-80. doi: 10.22038/jreh.2021.7068.

47 Masoud G, Abbass A, Abaza A, Hazzah W. Bacteriological quality of some swimming pools in Alexandria with special reference to Staphylococcus aureus. Environ Monit Assess. 2016;188(7):412. doi: 10.1007/s10661-016-5427-8. PMID: 27312255.

48 Leoni E, Legnani P, Guberti E, Masotti A. Risk of infection associated with microbiological quality of public swimming pools in Bologna, Italy. Public Health. 1999;113(5):227-32. doi: 10.1038/sj.ph.1900569. PMID: 10557116.

49 Abd El-Salam MM. Assessment of water quality of some swimming pools: a case study in Alexandria, Egypt. Environmental monitoring and assessment. 2012;184:7395-406. PMID: 22258741. doi: 10.1007/s10661-011-2508-6.