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Investigation of Water Quality and Molecular Analysis (16s rRNA) of Bacterial Community Structure in a Rural Geothermal Spring: Ucler Hill (Azat Village), Kars, Türkiye

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Abstract: This study was carried out to evaluate the chemical and microbiological properties of a natural water source located in Azat Village (Ucler hill) of Kars province and used as drinking water by the surrounding villagers. Water samples taken from three different sampling points were analyzed for pH, electrical conductivity, total hardness, alkalinity, nitrogenous compounds (NO_2^-, NO_3^-, NH_4^+), metal contents (Fe, Cu, Al, Pb), sulfate and chloride levels. The data obtained were compared with World Health Organization (WHO) drinking water standards and one-sample t-test was applied for each parameter. Statistically significant differences were found in total hardness, NO_3^-, Fe, Cu, SO_4^{2-} and Cl^- parameters ($p < 0.05$), while other parameters were found to be compatible with WHO reference values. In microbiological analyses, a total of eight different bacterial species were isolated by culture-dependent methods. These species were identified by 16S rRNA gene sequencing analysis and all of them were determined to be non-pathogenic environmental microorganisms. The identified species included *Microbacterium* spp., *Bacillus subtilis*, *Corynebacterium* spp., *Lactobacillus* spp., *Rhodococcus* spp., *Pseudomonas putida*, *Leuconostoc* spp. and *Aeromonas* spp. Although some species have the potential to be opportunistic pathogens, the overall microbiological profile was considered to be within acceptable limits for natural sources.

Kırsal Bir Jeotermal Kaynakta Su Kalitesi ve Moleküler Analiz (16s rRNA) ile Bakteriyel Topluluk Yapısının İncelenmesi: Üçler Tepesi (Azat Köyü), Kars, Türkiye

Anahtar Kelimeler
Doğal içme suyu kaynağı,
Mikrobiyal çeşitlilik,
Kırsal su güvenliği,
Kimyasal su kalitesi,
16S rRNA dizi analizi

Öz: Bu çalışma, Kars ilinin Azat Köyü'nde (Üçler tepesi) bulunan ve çevre köylüler tarafından içme suyu olarak kullanılan doğal bir su kaynağının kimyasal ve mikrobiyolojik özelliklerini değerlendirmek amacıyla gerçekleştirilmiştir. Üç farklı örnekleme noktasından alınan su örnekleri pH, elektriksel iletkenlik, toplam sertlik, alkalinite, azotlu bileşikler (NO_2^-, NO_3^-, NH_4^+), metal içerikleri (Fe, Cu, Al, Pb), sülfat ve klorür seviyeleri açısından analiz edilmiştir. Elde edilen veriler Dünya Sağlık Örgütü (WHO) içme suyu standartları ile karşılaştırılmış ve her parametre için tek örneklem t-testi uygulanmıştır. Toplam sertlik, NO_3^-, Fe, Cu, SO_4^{2-} ve Cl^- parametrelerinde istatistiksel olarak anlamlı farklılıklar bulunurken ($p<0.05$), diğer parametreler WHO referans değerleriyle uyumlu bulunmuştur. Mikrobiyolojik analizlerde, kültüre bağlı yöntemlerle toplam sekiz farklı bakteri türü izole edilmiştir. Bu izolatlar 16S rRNA gen dizileme analizi ile tanımlanmış ve patojen olmayan çevresel mikroorganizmalar olduğu belirlenmiştir. Tanımlanan türler arasında *Microbacterium* spp., *Bacillus subtilis*, *Corynebacterium* spp., *Lactobacillus* spp., *Rhodococcus* spp., *Pseudomonas* putida, *Leuconostoc* spp. ve *Aeromonas* spp. yer almaktadır. Bazı türler fırsatçı patojen olma potansiyeline sahip olsa da, genel mikrobiyolojik profilin doğal kaynaklar için kabul edilebilir sınırlar içinde olduğu düşünülmüştür.

1. INTRODUCTION

Freshwater resources are vital for both drinking and agricultural irrigation, especially for rural communities [1]. However, the sustainable and safe use of these resources is directly related not only to their quantity but also to their microbiological and chemical quality [2, 3]. Especially Kars province, which is located in the east of Turkey and has various geological structures, has a significant potential in terms of natural spring waters [4]. Azat village and its surroundings, which are discussed in this study, offer a remarkable research area due to their geographical location and hydrogeological characteristics. Ucler locality, which is close to the central district of Kars, where Azat Village is located, has a volcanic crater form with its topographical structure. Satellite images and field observations show that this region bears the traces of volcanic activities that occurred in the historical process. The flattened area in the center of the crater structure and the surrounding elevations suggest that the hydrogeochemical processes in this region are effective on the groundwater regime (Figure 1). In areas with volcanic rocks, the interaction of water with various minerals during its passage through the subsurface may create an environment that is both enriched in terms of ionic composition and suitable for the development of unique microbial communities [5].
In this context, remarkable differences can be observed in physicochemical parameters such as pH, conductivity and organic matter content as well as metals such as iron, manganese, sulfate and aluminum in water resources of volcanic origin. In fact, previous studies in these regions have reported critical values for human health, especially excessive iron and copper content, high ammonium and nitrite levels [6-8]. However, the fact that natural springs in such geological structures have been preferred by the public as drinking and utility water for many years brings with it the risk of ignoring possible microbiological threats.

From a microbiological point of view, natural mineralized waters formed by volcanic structures allow the development of bacterial communities adapted to harsh environmental conditions such as extreme temperature, pH changes and high ionic stress [9, 10]. These microorganisms are mostly saprophytes, but occasionally pathogenic species such as members of *Enterobacteriaceae*, *Pseudomonas* spp., *Staphylococcus* spp. and *Listeria* spp. have been reported [4, 11-13]. These bacteria may pose a risk of infection, especially for immunocompromised individuals and animals [12, 14]. In addition, the antibiotic resistance and heavy metal tolerance of bacteria also indicate the potential for significant public health problems in the long-term use of such resources.

This study aims to investigate both chemical parameters and microorganisms isolated by culture-based methods from a natural water source located in Azat Village of Kars province and used directly as a drinking water source by local people. In addition, identification of the bacterial isolates obtained at the molecular level by 16S rRNA gene region sequencing analysis will be carried out. Thus, the possible microbial risks, metal tolerance, diversity level and public health impact of natural water resources in the region will be revealed in a multidimensional way.

The results of this study will provide important information not only for the Kars region but also for the identification and control of environmental microorganisms that may threaten public health in rural areas with similar geological structures.

2. MATERIAL AND METHOD

2.1. Biological Material

The water samples analyzed in this study were collected from a natural water source located within the borders of Azat Village in the central district of Kars province, which is widely preferred by the local people for drinking and utility water. The spring is located in the center of an old volcanic structure at approximately 40.511855 °N latitude and 43.139596 °D longitude (Figure 1). This geological structure is characterized by its crater-like morphology and offers unique conditions for the mineral content and microbial load of the water. The sampling points on the map are marked in Figure 1. Sampling was conducted in June 2025 under moderate weather conditions; the season, date, time, and local observations (including limited animal activity around the source and moderate water flow) were recorded to provide context for the one-time sampling. A total of three different sampling points were identified; these points were taken from the main outlet near the center of the water source, from the middle zone downstream and from the expansion area approximately 50 meters beyond the outlet. Two samples were taken from each point, one from the surface (0-10 cm) and one from the bottom (20-30 cm), for a total of six water samples. Water samples were filled into sterile glass bottles and sealed to minimize contact with air during sampling [15]. The samples were transported to the laboratory of the Department of Bioengineering, Kafkas University within a maximum of 6 hours using ice-reinforced portable boxes. A constant temperature of less than 4°C was maintained during transportation of the samples, and the box was kept sterile to prevent external contamination. All samples were immediately separated for microbiological and chemical analyses upon arrival at the laboratory. During field studies, physical parameters such as water temperature, pH and electrical conductivity were measured and recorded instantly in the field with portable measuring devices [16]. At the same time, sampling coordinates, environmental observations and risk factors (animal mobility, agricultural activities, soil structure, vegetation cover, etc.) were documented in detail with a GPS device.

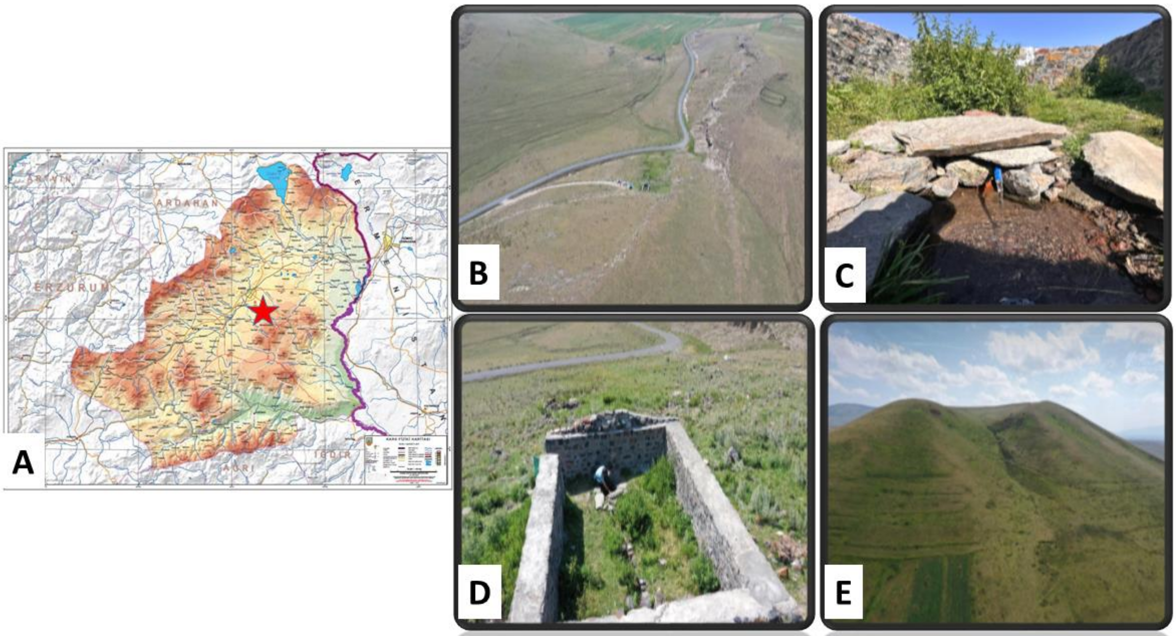

Figure 1. Visual components of the study area. (A) Location of the water source (indicated by the red star) on the physical map of Turkey-Kars province obtained from the General Directorate of Mapping [17]. (B) General view of the vicinity of the water source taken by drone from an altitude of approximately 350 m. (C) Stone structure and water outlet point in the inner region used directly as drinking water by local people. (D) View of the water source from upper elevations and surrounding settlement structure. (E) Topographic view of the northern slope of the volcanic mountain where the water source is located

2.2. Water Quality Assessment Based on Chemical Indicators

Within the scope of the research, chemical evaluation of water samples collected from three different points in Azat Village was carried out using internationally accepted standard analysis methods. In these analyses, physical parameters such as pH, conductivity, total hardness and alkalinity were first measured; then the levels of chemical components such as nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), iron (Fe), aluminum (Al), copper (Cu), lead (Pb), sulfate (SO_4^{2-}) and chloride (Cl^-) were determined. All analyses were carried out in a laboratory approved by the Ministry of Agriculture and Forestry, based on Turkish Standards Institute (TSE) and World Health Organization (WHO) drinking water quality criteria [18]. The data obtained were presented in a tabular form to allow comparison of the parameters with the limit values and their compliance status was also evaluated. Thus, it was objectively revealed whether the water source carries a potential risk in terms of potability. All chemical analyses were performed in an accredited laboratory in accordance with TSE and WHO standard protocols [18]. Device calibration, blind sample and standard use, and repeat measurements were performed to ensure quality assurance/quality control (QA/QC) and the reliability of the results.

2.3. Isolation and Cultivation of Environmentally Brought Bacteria

Bacterial isolation from water samples was carried out using culture-based classical microbiological methods. After the samples arrived at the laboratory, they were subjected to serial dilutions from 10^{-1} to 10^{-6} under aseptic conditions [15]. Each dilution was inoculated into Nutrient Agar (NA) medium by the surface spreading method using sterile pipettes. Incubation in Petri dishes was performed at 37°C under aerobic conditions for 24 to 72 hours. Colonies exhibiting different colony morphologies were selected and reseeded by drawing method to obtain pure culture. The purified isolates were stored at -20 °C in liquid medium containing 15% glycerol for storage as stock cultures [19]. This approach provides a baseline for the preliminary identification of the dominant bacterial species naturally found in environmental water sources. Isolates were subjected to preliminary classification by observing colony morphology, color, size and margin structure. The pure cultures obtained were transferred to the next stages to be used for further molecular analyses [20].

2.4. DNA Preparation Protocol for Molecular Analyses

Genomic DNA extraction from pure cultures obtained as a result of bacterial isolations was carried out based on the phenol-chloroform-based classical method described in the literature [21]. Firstly, the isolates were incubated in liquid medium overnight and then the cells were precipitated by centrifugation. The cell pellets obtained were treated with lysis buffer and cell walls were lysed. Proteinase K and SDS were applied to remove protein structures, then DNA was extracted with phenol: chloroform: isoamyl alcohol (25:24:1). DNA samples precipitated with isopropanol were washed with 70% ethanol, dried and dissolved in TE buffer [15]. The purity and integrity of the DNA were checked by NanoDrop spectrophotometer and 1% agarose gel electrophoresis [22, 23]. Suitable samples were stored for further molecular analysis.

2.5. 16S rRNA profiling of culture-based isolates

The high purity genomic DNA samples obtained were subjected to PCR analysis targeting the 16S rRNA gene region for bacterial species identification. Amplification was performed using universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT GTT ACG ACT T-3′) [24]. The PCR reaction was prepared in a total volume of 25 µL and included Taq DNA polymerase, dNTP mix, $MgCl_2$, primers and template DNA. Reactions were run in thermal cycles using standard temperature cycles and the PCR products obtained were visualized and verified on a 1% agarose gel [4]. Cleaned amplicons were sequenced by Sanger sequencing using a commercial sequencing service. The sequences were converted to FASTA format and analyzed by BLASTn searches in the NCBI GenBank database for species-level identification. Matches with 98% or more similarity were considered for taxonomic matching.

2.6. Assessment of Molecular Analysis

To reveal the similarities between the bacterial species isolated in this study, sequences belonging to the 16S rRNA gene region were amplified by PCR. The sequence analysis results were organized in FASTA format, and the closest matching culture reference species were determined by BLASTn search [25]. Sequences from environmental or undescribed species were not included in the evaluation.

2.7. Statistical Analyses

The chemical parameter data obtained in the study were evaluated in comparison with the drinking water standards recommended by the World Health Organization (WHO) [18]. Three repeated measurements were made for each parameter and mean values were calculated together with standard deviations. One-sample t-test was performed on the obtained data to evaluate whether the measured values showed a statistically significant difference with the reference limits. The significance level was accepted as $p<0.05$. All calculations were carried out using Microsoft Excel and IBM SPSS Statistics software and the results were supported by relevant graphs and tables [26]. This numerical evaluation aimed to increase the scientific reliability of both chemical and microbiological results.

3. RESULTS

3.1. General Characteristics of the Sampling Area and Water Quality Indicators

This study was carried out in a natural water source located in Azat Village of Kars province and used as drinking water by the public. The water temperature of the spring was approximately 35 °C and the pH value was measured as 7.4. The water is in the neutral pH range and has natural spring characteristics. In the chemical analysis, 13 different parameters were evaluated. All of these parameters such as pH, conductivity, total hardness, alkalinity, NO_2^-, NO_3^-, NH_4^+, Al, Pb, SO_4^{2-} and Cl^- were found to comply with the limit values set by the World Health Organization for drinking water. However, Fe (0.32 mg/L) and Cu (2.05 mg/L) levels were slightly above the limit values (Fe ≤ 0.3, Cu ≤ 2.0). Therefore, these two parameters were evaluated as "above the limit, therefore non-compliant". This can be explained by the effect of the geological structure through which the water passes. Overall, the chemical quality of the Azat Village water source is largely suitable for drinking (Table 1).

Table 1. Mean values, standard deviations, WHO drinking water limit values and compliance status of chemical parameters measured in samples taken from the Azat Village water source.

Parameter	Average Value ($\bar{X}$)	Standard Deviation (±)	WHO Limit	Compliance
pH	7.4	0.1	6.5 – 8.5	Compliance
E.C. (mS/cm)	2.4	0.05	<2.5	Compliance
Total Hardness (mg/L $CaCO_3$)	290.0	10.0	0 – 500	Compliance
Alkalinity (mg/L)	180.0	8.0	0 – 200	Compliance
NO_2^- (mg/L)	0.48	0.03	≤0.5	Compliance
NO_3^- (mg/L)	45.5	1.5	≤50	Compliance
NH_4^+ (mg/L)	0.48	0.02	≤0.5	Compliance
Fe (mg/L)	0.32	0.01	≤0.3	**Non-compliant**
Al (mg/L)	0.19	0.01	≤0.2	Compliance
Cu (mg/L)	2.05	0.04	≤2.0	**Non-compliant**
Pb (mg/L)	0.009	0.002	≤0.01	Compliance
SO_4^{2-} (mg/L)	120.0	4.3	≤250	Compliance
Cl^- (mg/L)	41.0	1.2	≤250	Compliance

3.2. Morphological Characterization of Isolates

The bacteria were isolated from water samples and analyzed by Gram staining method. In microscopic analysis, mostly Gram positive rod and cocci form bacteria were observed (Figure 2 and Table 2). All of these bacteria are among the non-pathogenic species commonly found in drinking and environmental waters in the literature. As a result of the morphological examinations, bacteria similar to the following were identified among the identified species: *Microbacterium spp., Bacillus subtilis, Corynebacterium spp., Lactobacillus spp., Rhodococcus spp., Pseudomonas putida, Leuconostoc spp. and Aeromonas spp.* These species are described as generally harmless aquatic bacteria with high environmental tolerance in natural waters and do not show pathogenicity.

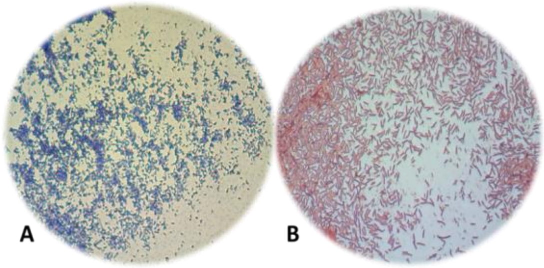

Figure 2. Gram staining micrographs of isolated bacterial species. **(A)** Gram-positive, short rod morphology *Microbacterium* spp. AZ1 is characterized by blue-purple stained cell structure. **(B)** Microscopic image of a Gram-negative, pink-colored long rod-shaped *Pseudomonas putida* AZ6. Both images were taken with immersion oil at 1000× magnification

3.3. 16S rRNA Gene Regions of the Isolates

In order to identify the bacteria isolated in the study at species level, genomic DNA of each isolate was obtained following appropriate protocols. Then, the 16S rRNA gene region was successfully amplified and sequenced using universal primers (Figure 3). The sequences obtained were compared with the NCBI GenBank database and species with 98% or more similarity were identified (Table 2). According to the molecular identification results, a total of 8 different species were identified. The majority of these species are non-pathogenic bacteria that are frequently encountered as environmental saprophytes in natural drinking water sources. These results indicate that the bacteria present in the water source are largely of environmental origin and non-pathogenic in character. These molecular level analyses provided an important overview of the microbial content of the water.

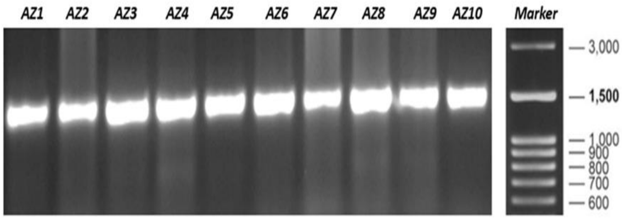

Figure 3. Agarose gel image of PCR amplification of the 16S rRNA gene region of bacteria isolated from water samples from Azat Village. The bands approximately 1500 bp long were observed in all isolates.

Table 2. Optimum pH and temperature ranges of bacterial species isolated from the Azat Village water source. All isolates grew in the pH range of 7.0–7.5, and temperature tolerances varied among species.

Isolate	Identified	pH	Temperature Range / T_{opt} (°C)	Gram Staining	Form
AZ1	*Microbacterium* spp.	7.0–7.5	20–45 / 37	+	*Bacilli*
AZ2	*Bacillus subtilis*	7.0–7.5	35–50 / 40	+	*Bacilli*
AZ3	*Corynebacterium* spp.	7.0–7.5	20–40 / 30	+	*Bacilli* (irregular form)
AZ4	*Lactobacillus* spp.	7.0–7.5	20–45 / 37	+	*Bacilli*
AZ5	*Rhodococcus* spp.	7.0–7.5	25–45 / 37	+	*Cocco bacil*
AZ6	*Pseudomonas putida*	7.0–7.5	25–45 / 37	-	*Bacilli*
AZ7	*Leuconostoc* spp.	7.0–7.5	15–35 / 30	+	*Cocci*
AZ8	*Aeromonas* spp.	7.0–7.5	20–42 / 35	-	*Bacilli*
AZ9	*Bacillus* sp.	7.0–7.5	35–50 / 40	+	*Bacilli*
AZ10	*Pseudomonas* sp.	7.0–7.5	25–45 / 37	-	*Bacilli*

3.4. The Chemical Parameters of the Water Samples

The comparative analysis of the chemical parameters of the water samples taken from Azat Village with the drinking water limit values determined by the World Health Organization (WHO) was carried out by one-sample t-test. Three repeated measurements were taken for each parameter and statistical validity was tested by calculating the mean value and standard deviation. The Total Hardness, expressed as mg/L $CaCO_3$, was found to be significantly less than the WHO upper limit of 500 mg/L in the cases under concern ($p = 0.0004$). This indicates the natural low hardness level of water in the region but within acceptance. Except for these, parameters such as pH, conductivity, alkalinity, NO_2^-, NO_3^-, NH_4^+, Al, Pb, SO_4^{2-}, and Cl^- showed mean values which were not significantly different from the WHO limit ($p > 0.05$). The results imply that, in general, the chemical composition of the water is safe for drinking purposes. However, the measured mean values of Fe (mg/L) and Cu (mg/L) parameters were slightly above the WHO recommended limit values, and these differences were found to be statistically significant ($p < 0.05$).

Table 3. One-sample t-test results for comparing chemical parameters obtained from Azat Village water samples with World Health Organization (WHO) drinking water standard values. Mean value, standard deviation, reference value, t-statistic, and p-value are given for each parameter, and significance was assessed at $p < 0.05$.

Parameter	Average	Standard Deviation	WHO Reference	t-statistic	p-value
pH	7.4	0.1	7.5	0.121	0.9151
Electrical Conductivity (mS/cm)	2.4	0.05	2.5	-2.41	0.1376
Total Hardness (mg/L $CaCO_3$)	290.0	10.0	500.0	-50.483	0.0004
Alkalinity (mg/L)	180.0	8.0	200.0	-3.675	0.0667
NO_2^- (mg/L)	0.48	0.03	0.5	0.964	0.4368
NO_3^- (mg/L)	45.5	1.5	50.0	-4.94	0.0386
NH_4^+ (mg/L)	0.48	0.02	0.5	0.121	0.9151
Fe (mg/L)	0.32	0.01	0.3	7.711	0.0164
Al (mg/L)	0.19	0.01	0.2	0.121	0.9151
Cu (mg/L)	2.05	0.04	2.0	5.813	0.0283
Pb (mg/L)	0.009	0.002	0.01	1.386	0.3001
SO_4^{2-} (mg/L)	120.0	4.3	250.0	-73.843	0.0002
Cl^- (mg/L)	41.0	1.2	250.0	-438.024	0.0

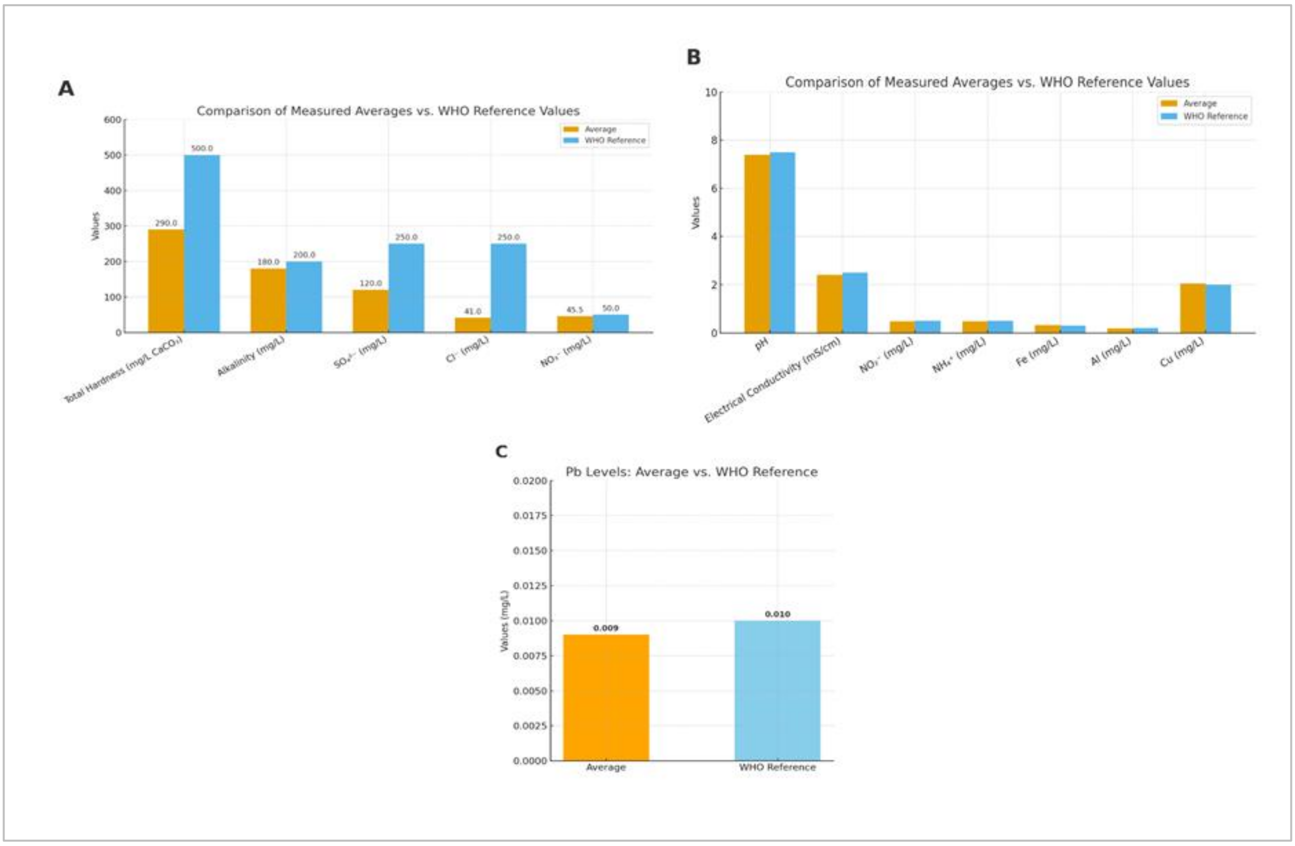

Figure 4. Comparison of average measured values of chemical parameters with the WHO drinking water standards. **(A)** Total Hardness, Alkalinity, SO_4^{2-}, Cl^-, and NO_3^- concentrations; **(B)** pH, Electrical Conductivity, NO_2^-, NH_4^+, Fe, Al, and Cu values; **(C)** Pb levels.

4. DISCUSSION

In this study, a natural water source located in Azat Village of Kars province and preferred by local people for drinking and utilization purposes was evaluated chemically and microbiologically. The results show that the spring water is safe for drinking to a large extent, but some parameters have reached limits that require caution. In this context, the results are important not only for regional public health but also for shaping rural water security policies.

From the chemical analyses, 11 out of 13 parameters fell within the prescribed limits for potable water as per WHO standards [18]. Thus, water specimens were under permissible limits for pH, conductivity, total hardness, alkalinity, nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), aluminum (Al), lead (Pb), sulfate (SO_4^{2-}), and chloride (Cl^-). This indicates that the water in Azat Village comes from a geologically stable composition and is insulated from sources of external contamination. Especially nitrate and ammonium are below the limits, which means that the source is comparatively protected from agrarian pollution and livestock effluent [27, 28].
Here is a noteworthy observation: Whereas Fe and Cu levels were ever so slightly above the WHO limits, the mean value for iron is 0.32 mg/L against WHO limit of 0.3 mg/L and 2.05 mg/L for copper, while the limit value was 2.0 mg/L [18]. The one-sample t-test showed that the differences for both parameters were statistically significant ($p < 0.05$). Still, these slight differences warrant long-term monitoring. This result may be attributed to the low measurement variations and limited number of samples. The fact that both parameters were very close to the limit indicates that they should be monitored carefully, especially for long-term use. On the contrary, the solubility of these metals in water might be associated with the rock structure lying around the source and some geothermal activity effects [29]. Copper, in particular, is among the elements that can pass into water from soils and rocks of volcanic origin [30]. As a result of microbiological analyses, a total of eight different bacterial species were isolated by culture-based methods and identified at molecular level by 16S rRNA gene sequencing. These bacteria include *Microbacterium* spp., *Bacillus* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Rhodococcus* spp., *Pseudomonas* spp., *Leuconostoc* spp. and *Aeromonas* spp. All these taxa are delineated in the scholarly corpus as saprophytic and generally non-pathogenic microorganisms associated with environmental aquatic systems [4, 31-34]. These observations, corroborated by Gram staining and morphological assessments, suggest that the microbial quality of the water is at an acceptable standard. Nevertheless, the identification of opportunistic pathogenic taxa such as *Aeromonas* and *Pseudomonas*, even at minimal concentrations, is significant as they may present a potential hazard for individuals with compromised immune systems [35-37].

An important contribution that is being made by this study is the detailed examination of a remote water source existing in a geologically and climatically unique geography of Kars, chemically, and microbiologically.

One thing that the literature report clearly states is that the number of scientific data on natural water resources in and around Kars is very limited. Therefore, the product of this research will help raise awareness among the local people in the context of water safety, hygiene, and public health, apart from providing scientific input to local governments for preparing monitoring and improvement strategies. On the other hand, the study also faces some constraints. It remained as a single episode sampling with limited repetitions. Considering the effects of seasonal differences on chemical and microbiological parameters, it is recommended that such studies be repeated periodically throughout the year in the future. In addition, revealing a wider microbial diversity through non-culture-dependent methods such as metagenomic analysis will provide a more comprehensive perspective on the microbiological safety of water.

5. CONCLUSIONS

This study evaluated the chemical and microbiological quality of the natural drinking water source in Azat Village, Kars province. As a result of the analyses, it was determined that most chemical parameters were in compliance with the World Health Organization (WHO) drinking water standards. However, iron and copper levels were very close to the limit values and it should be emphasized that they should be monitored regularly for long-term use.

A total of eight bacterial species were identified in the microbiological analyses, most of which were environmental and non-pathogenic. However, the presence of some opportunistic species reveals a situation that needs to be carefully monitored.

In conclusion, although the Azat Village water source is generally potable, periodic chemical and microbiological controls are recommended, taking into account seasonal changes. In the future, more comprehensive microbial diversity screening with metagenomic analysis will make important contributions to water safety. In addition, the data obtained are also instructive for the studies to be carried out for other natural resources in the region.

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REFERENCES

[1] Mishra, R.K., Fresh water availability and its global challenge, British Journal of Multidisciplinary and Advanced Studies 4(3) 2023; 1-78.
[2] Till, D., G. McBride, A. Ball, K. Taylor, E. Pyle, Large-scale freshwater microbiological study: rationale, results and risks, Journal of Water and Health 6(4) 2008; 443-460.
[3] Verma, S., S. Verma, R. Ramakant, V. Pandey, A. Verma, Comprehensive Assessment of Physico-Chemical and Biological Parameters in Water Quality Monitoring: A Review of Contaminants, Indicators, and Health Impacts, International Journal of Horticulture, Agriculture and Food science 9(2) 2025; 611019.
[4] Ulucay, O., Potential Health Risks of the Çakmak Village Thermal Spring (Turkey, Kars) for Grazing Animals: Characterization of Bacterial Communities and Water Quality, Journal of Environmental Chemical Engineering 2025; 118059.
[5] Reysenbach, A.-L., E. St. John, J. Meneghin, G.E. Flores, M. Podar, N. Dombrowski, A. Spang, S. L'Haridon, S.E. Humphris, C.E.J. De Ronde, Complex subsurface hydrothermal fluid mixing at a submarine arc volcano supports distinct and highly diverse microbial communities, Proceedings of the National Academy of Sciences 117(51) 2020; 32627-32638.
[6] Uluçay, O., A. Görmez, C. Öziç, Determination of Physical, Chemical Properties of the Hot Springs Waters and Comparison of Bacteria: Hot Springs in the East and Southeast Anatolia Region of Turkey, Journal of Agriculture 4(1) 2021; 24-29.
[7] Hamilton, T.L., E. Koonce, A. Howells, J.R. Havig, T. Jewell, J.R. de la Torre, J.W. Peters, E.S. Boyd, Competition for ammonia influences the structure of chemotrophic communities in geothermal springs, Appl Environ Microb 80(2) 2014; 653-661.
[8] Dippong, T., M.-A. Resz, Heavy metal contamination assessment and potential human health risk of water quality of lakes situated in the protected area of Tisa, Romania, Heliyon 10(7) 2024.
[9] Lynch, R.C., A.J. King, M.E. Farías, P. Sowell, C. Vitry, S.K. Schmidt, The potential for microbial life in the highest-elevation (> 6000 masl) mineral soils of the Atacama region, Journal of Geophysical Research: Biogeosciences 117(G2) 2012.
[10] Smrhova, T., K. Jani, P. Pajer, G. Kapinusova, T. Vylita, J. Suman, M. Strejcek, O. Uhlik, Prokaryotes of renowned Karlovy Vary (Carlsbad) thermal springs: phylogenetic and cultivation analysis, Environmental Microbiome 17(1) 2022; 48.
[11] Arvanitidou, M., A. Papa, T.C. Constantinidis, V. Danielides, V. Katsouyannopoulos, The occurrence of Listeria spp. and Salmonella spp. in surface waters, Microbiological Research 152(4) 1997; 395-397.
[12] Ghilamicael, A.M., H.I. Boga, S.E. Anami, T. Mehari, N.L.M. Budambula, Potential human pathogenic bacteria in five hot springs in Eritrea revealed by next generation sequencing, PLoS One 13(3) 2018; e0194554.
[13] Rahel, C., R. Adriyani, H.A. Nurfitria, Health risk in hot springs: A literature review, Journal of Health Science and Prevention 5(2) 2021; 88-99.
[14] Deza, M.A., M. Araujo, M.J. Garrido, Inactivation of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa and Staphylococcus aureus on stainless steel and glass surfaces by neutral electrolysed water, Lett Appl Microbiol 40(5) 2005; 341-346.

[15] Ulucay, O., A. Gormez, C. Ozic, Identification, characterization and hydrolase producing performance of thermophilic bacteria: geothermal hot springs in the Eastern and Southeastern Anatolia Regions of Turkey, Antonie van Leeuwenhoek 2022; 1-18.
[16] Uluçay, O., & Öziç, C. (2025). Molecular identification and phylogenetic profiling of keratinolytic bacteria isolated from goose and chicken farms in a cold region of Türkiye. International Journal of Agriculture Environment and Food Sciences, 9(2), 436-445. https://doi.org/10.31015/2025.2.17
[17] Kars Maps, 2025. https://www.harita.gov.tr/urun/kars-fiziki-il-haritasi/382. (Accessed 25.06.2025 2025).
[18] Edition, F., Guidelines for drinking-water quality, WHO chronicle 38(4) 2011; 104-8.
[19] Oztas Gulmus, E., A. Gormez, Identification and characterization of novel thermophilic bacteria from hot springs, Erzurum, Turkey, Curr Microbiol 77(6) 2020; 979-987.
[20] Ahmadpour, A., Y. Ghosta, Z. Alavi, F. Alavi, L.M. Hamidi, P.H. Rampelotto, Morphological and Molecular Characterization of a New Section and Two New Species of Alternaria from Iran, Life 15(6) 2025; 870.
[21] Javadi, A., M. Shamaei, L.M. Ziazi, M. Pourabdollah, A. Dorudinia, S.M. Seyedmehdi, S. Karimi, Qualification study of two genomic DNA extraction methods in different clinical samples, Tanaffos 13(4) 2014; 41.
[22] Elkins, K.M., Determination of Quality and Quantity of DNA Using Agarose Gel Electrophoresis, Forensic DNA Biology. Oxford, USA: Elsevier Inc 2013; 53-57.
[23] Gurcan, K., F. Demirel, M. Tekin, S. Demirel, T. Akar, Molecular and agro-morphological characterization of ancient wheat landraces of turkey, Bmc Plant Biol 17(Suppl 1) 2017; 171.
[24] Chen, C., P.H. Qu, Q. Gu, B. Huang, W.Z. Zhang, X.P. Mu, L. Zhang, M.R. Chen, L.X. Wang, Y.J.Y. E Sm, Application of broad-spectrum PCR amplification and direct sequencing for identification of the infrequent bacterial cultures from clinical sources, targeting the bacterial 16S rRNA gene with universal primers, Chin J Lab Med 35 2012; 612-619.
[25] Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J.-P. Gayral, D. Raoult, 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates, Journal of clinical microbiology 38(10) 2000; 3623-3630.
[26] Meyers, L.S., G.C. Gamst, A.J. Guarino, Performing data analysis using IBM SPSS, John Wiley & Sons2013.
[27] Sahoo, P.K., K. Kim, M.A. Powell, Managing groundwater nitrate contamination from livestock farms: implication for nitrate management guidelines, Current Pollution Reports 2(3) 2016; 178-187.
[28] Lawniczak, A.E., J. Zbierska, B. Nowak, K. Achtenberg, A. Grześkowiak, K. Kanas, Impact of agriculture and land use on nitrate contamination in groundwater and running waters in central-west Poland, Environmental monitoring and assessment 188(3) 2016; 172.
[29] Crerar, D., S. Wood, S. Brantley, A. Bocarsly, Chemical controls on solubility of ore-forming minerals in hydrothermal solutions, The Canadian Mineralogist 23(3) 1985; 333-352.
[30] Zampella, M., P. Adamo, L. Caner, S. Petit, D. Righi, F. Terribile, Chromium and copper in micromorphological features and clay fractions of volcanic soils with andic properties, Geoderma 157(3-4) 2010; 185-195.
[31] Farrar Jr, W.E., Serious infections due to "non-pathogenic" organisms of the genus bacillus: Review of their status as pathogens, The American journal of medicine 34(1) 1963; 134-141.
[32] Hajmeer, M.N., D.Y.C. Fung, Infections with other bacteria, Foodborne Infections and Intoxications (3rd Ed.) Elsevier, UK 2006; 341-367.
[33] Oliveira, A., L.C. Oliveira, F. Aburjaile, L. Benevides, S. Tiwari, S.B. Jamal, A. Silva, H.C.P. Figueiredo, P. Ghosh, R.W. Portela, Insight of genus Corynebacterium: ascertaining the role of pathogenic and non-pathogenic species, Frontiers in microbiology 8 2017; 1937.
[34] Ivshina, I.B., M.S. Kuyukina, A.V. Krivoruchko, E.A. Tyumina, Responses to ecopollutants and pathogenization risks of saprotrophic Rhodococcus species, Pathogens 10(8) 2021; 974.
[35] Janda, J.M., S.L. Abbott, The genus Aeromonas: taxonomy, pathogenicity, and infection, Clinical microbiology reviews 23(1) 2010; 35-73.
[36] Fernández-Bravo, A., M.J. Figueras, An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity, Microorganisms 8(1) 2020; 129.
[37] De Bentzmann, S., P. Plésiat, The Pseudomonas aeruginosa opportunistic pathogen and human infections, Environmental microbiology 13(7) 2011; 1655-1665.