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Identification and Characterization of Environmental Bacteria Isolated in a Naturally Carbonated Spring in Türkiye: Chemical and Microbial Responses to Excessive Manganese Accumulation

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Abstract In this study, the chemical constitution and microbial heterogeneity of a natural soda water spring situated in Çerme Village, Turkey were examined. In the chemical assessments, it was ascertained that several parameters such as pH, calcium, magnesium, chloride, bicarbonate and total organic matter were in accordance with potable water criteria. However, it was determined that some elements such as manganese (0.83487 mg/L), nitrite, ammonium, iron, aluminum, copper and sulfur exceeded the limit values. A total of 12 bacteria were isolated using culture-based methods and these isolates were identified at species level. The bacteria that were isolated are composed of the genera *Staphylococcus*, *Kurthia*, *Micrococcus*, *Pseudomonas*, and *Listeria*. The Shannon diversity index ($H' = 1.86$) suggests that there is moderate microbial diversity. It is worth mentioning that the very high concentration of manganese seems to be the main factor in this spring ecosystem—the survival of metal-tolerant environmental bacteria is the result of selective pressure that is exerting strong influence. The findings from this research indicate the importance of periodical examination of natural water sources that have been traditionally used. In addition, these findings reveal significant patterns of bacterial diversity in areas contaminated with metals.

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1 Introduction

Freshwater ecosystems are significant from both the viewpoint of living things and human activities (Apostolaki et al., 2020). People frequently choose fresh water for their food, farming, and domestic drinking in the same areas (Sukanya & Joseph, 2024). It is well known that the microbiological and chemical properties of water sources are directly related to public health. Indeed, the World Health Organization (WHO) considers the monitoring of water sources and the provision of safe water to be an important factor for public health on a global scale (Edition, 2011). Depending on their geological characteristics, natural spring waters may have a high mineral content. In Turkey, a country with significant geothermal potential, sodic natural waters with high sodium bicarbonate content are common (Özgür and Büyükşahin). These sources are called soda water sources due to the minerals dissolved within them (Borzenko et al., 2018). These types of sources are used by some people because they are believed to have healing properties. Since these sources are found more in rural areas, they are less studied by authorities. It is known that the dilution of minerals such as bicarbonate, sodium, iron, and manganese in the aqueous phase

promotes the growth of certain microbial groups (Laanbroek, 1990). Such microbes become dominant because they can fight hard environmental challenges (Gadd, 2010). These microorganisms may include environmental bacteria such as *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Arthrobacter* spp. On the one hand, those bacteria participate in biogeochemical cycles and on the other hand, might have potential pathogens in them which could be a risk factor for the health of the public (Mhammedsharif & Kolo, 2023; Oluseyi Osunmakinde et al., 2019; Ulucay et al., 2022b). Previous studies have reported that metal-rich water environments usually have the presence of such bacterial genera as *Pseudomonas*, *Bacillus*, and *Micrococcus* because of their ability to survive in high concentrations of manganese, iron, and other heavy metals (Nnaji et al., 2024; Ramljak et al., 2025; Zhang et al., 2014). Most of the time, the current analyses using culture-dependent methods are crucial in providing the necessary information for the isolation and initial characterization of these microorganisms.

The microbiological diversity assessment still largely relies on culture-dependent methods, which also enable the isolation of specific bacterial groups and the obtaining of pure cultures (Lagier et al., 2015). Colony morphology-based preliminary assessments give initial data for the understanding of microorganisms' basic physiological traits (El Ahmadi et al., 2025; Sousa et al., 2013). In addition, phenotypic observations such as color, growth pattern, colony margin structure and surface characteristics become a great help in determining the next molecular analysis. The scenario of the natural lye water source of Çerme Village in Kars, Turkey is that the locals are using it as drinking water, but no scientific data is available regarding its microbiological and chemical properties. The study area is located in a mineral-rich region characterized by volcanic sediments and carbonate-rich formations. The region also affects the natural mineral load of local water sources.

This points to a significant lack of information, especially in terms of assessing the safe use of the water source. In addition, this water source is also used by many cattle and sheep in the region to meet their water needs while grazing on pastures. Such natural water sources may also play a role in potential pathogen transmission between animals and humans. The high altitude in the region (~1778 m) and the natural geological structure are among the environmental factors that may be determinant on the microbiological content of this water source. In addition, water parameters such as temperature, pH and dissolved ion content are among the main environmental determinants that shape microbial diversity.

In the literature, it has been reported that such water sources may be rich in iron, manganese and sulphate and therefore various metal tolerant microorganisms, especially iron-oxidizing bacteria, may develop (Nnaji et al., 2024; Roy et al., 2024; Sun et al., 2025). In similar studies, it has been shown that most of the bacteria isolated from natural spring waters are saprophytic species adapted to the environment, but potential pathogens can also be isolated from time to time. Especially members of *Enterobacteriaceae*, some *Staphylococcus* species and rarely anaerobes such as *Clostridium* can be detected in such natural environments (Mangoma et al., 2024).

However, it is also important to evaluate the characteristics of the isolated microorganisms such as their environmental functions, antibiotic resistance and metal tolerance for the long-term utilization of these resources. In this direction, in this study, preliminary identification of microorganisms isolated from water samples taken from a soda water source located in Çerme Village by culture-dependent methods will be carried out. In addition, it is aimed to contribute to the understanding of the effects of environmental factors on microbial diversity by measuring temperature, pH and other basic chemical parameters of the water source. This study aims to make a unique contribution to the scientific evaluation of traditionally used water sources, especially in rural areas. The reason for selecting this source is that it is still preferred as drinking and domestic water in the region and that preliminary measurements revealed a remarkably high manganese level. In Turkey, studies evaluating both the chemical composition and microbial community structure of natural gas sources are quite limited. Furthermore, research demonstrating the effect of manganese on microbial selection is also insufficient. Therefore, the Çerme source constitutes an important ecosystem that should be studied due to its high metal content and traditional use.

2 Materials and Methods

2.1 Biological Material

The water utilized for the investigation was procured from a geothermal spring situated at an elevation of roughly 1,778 m above sea level within the confines of Çerme Village in the Kars province (Fig. 1). The water from the locality was conveyed to the Kars State Hydraulic Works Water Analysis Laboratory for analytical examination. Samples were collected in July 2024, during the summer season, in the morning hours (09:00–10:00). During sampling, the ambient temperature was approximately 28 °C and the weather was clear. Water temperature and pH values were measured in situ at the source location. Approximately 500 mL water sample has been collected for microbiological and chemical analysis.

2.2 Investigation of Chemical Properties of Water Resource

In this study, various physico-chemical parameters including pH, TDS, alkalinity, calcium (Ca), magnesium (Mg), chloride (Cl), bicarbonate (HCO_3^-), total organic carbon, nitrite (NO_2), ammonium (NH_4), nitrate (NO_3), iron (Fe), aluminum (Al), copper (Cu), lead (Pb), sulfur (S), manganese (Mn) of the natural soda waters procured from Çerme Village were analyzed (Agilent 7850 ICP-MS and Thermo iCAP TQ ICP-MS). Within the scope of these analyses, comprehensive values were compared with the World Health Organization (WHO) and Turkish drinking water standards; some parameters, such as manganese, nitrite, ammonium, iron, aluminum, copper, lead, and sulfur, were found to be above the limit values. During the evaluations, the standards recommended by the World Health Organization for drinking water quality were taken into account and internationally valid methods were used as the basis in the analysis process (Edition, 2011).

2.3 Microbiological Isolation and Culture Conditions

In this study, water and mud samples were collected from a natural soda spring located in Çerme Village (40.456838°N, 42.973363°E), Kars Province. Sampling was carried out in places that had the least environmental degradation. Sterile thermal bottles, glass flasks were used for the collection of samples and the laboratory received them on the same day. The gathered samples were transferred to the laboratory within a period of no more than 2 h in ice-pack transport containers, under protected conditions to reduce light exposure to a minimum. In the laboratory, water samples were transferred to sterile glass containers

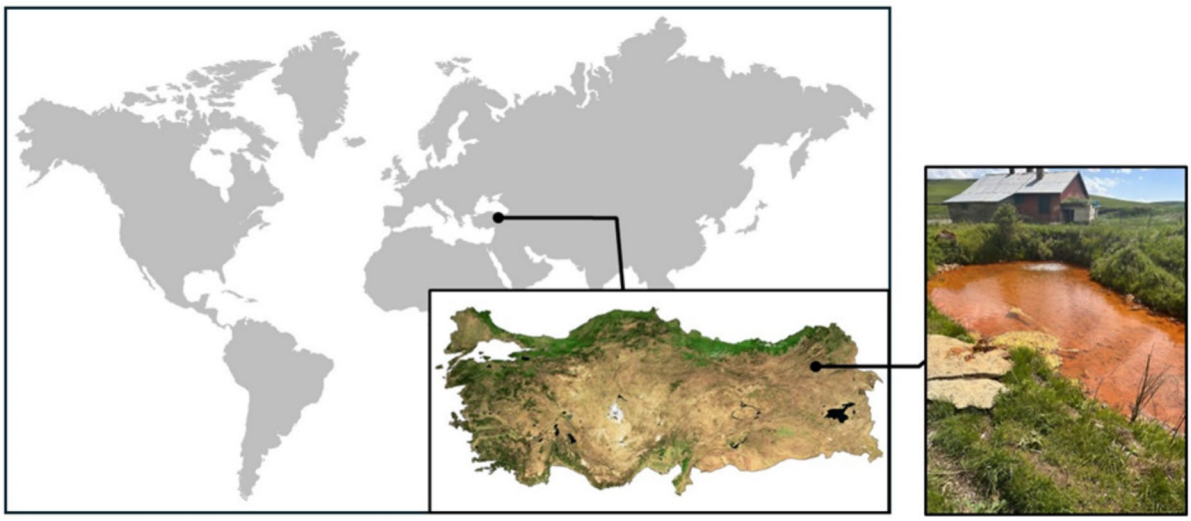


Fig. 1 The soda water source analyzed within the scope of the study was obtained from a natural outlet located at an altitude of approximately 1778 m above sea level within the borders of Çerme Village in Kars province Map source: GISGeopraphy (2025)

and diluted with physiological saline containing 0.85% sodium chloride (NaCl). Bacterial inoculation was achieved by applying different dilutions (Ulucay et al., 2022b).

Collected water samples were inoculated directly onto Nutrient Agar (NA) by spreading 0.1 mL of the culture medium. Sludge samples were prepared as serial dilutions (10^{-1}–10^{-6}) and streaked onto NA medium. Bacterial inoculations were incubated at 37 °C; the incubation period varied between 24 and 72 h, depending on the growth rate of the isolates. Purification was performed by selecting single colonies from the different colony types that developed in the cultures, and these pure colonies were prepared as streak cultures for stocking. Those isolates should be assigned identifiers for their regions. Such pure cultures were necessary for any other study detailing the isolation of DNA and evaluation of morphological features (Ulucay, 2025).

2.4 Genomic DNA Isolation from Bacterial Isolates

After cultivating bacterial isolates, the genomic DNA of those isolates was extracted based on the methods described in earlier publications with some modifications (Mijnendonckx et al., 2024). First, isolates were incubated in broth, then sedimented by centrifugation, and cell pellets were dissolved in lysis buffer. The cells were lysed by incubating them with lysozyme, following which Proteinase K was supplemented along with sodium dodecyl sulfate for protein degradation. DNA was isolated through phenol–chloroform extraction and precipitated with isopropanol as it became free from other cellular components. The precipitated DNA was then washed with 70% ethanol, air-dried, and finally dissolved in an appropriate volume of nuclease-free water. DNA quality and quantity were confirmed by means of agarose gel electrophoresis and spectrophotometry (Ulucay et al., 2022a).

2.5 16S rRNA Sequencing

The purity and concentration of bacterial DNA samples were estimated by NanoDrop. The PCR was carried out for the 16S rRNA gene region using universal primers 27F and 1492R. The 16S rRNA gene amplifications of the isolated 26 isolates were conducted by means of universal primers (Forward Primer: 27F = 5′-AGA GTT TGA TCC TGG CTC AG-3′, Reverse Primer: 1492R = 5′-GGT TAC CTT GTT ACG ACT T-3′). The final volume of the reaction was 25 µL. The mixture included a combination of Taq polymerase, buffer solution, $MgCl_2$, dNTP, primers, genomic DNA, and sterile purified water. PCR was conducted in a GenePro Thermal Cycler, after specific temperature cycles were applied (Ulucay, 2025). The resulting products on a 1% agarose gel to check if we had the right bands and to cut them out for purification (Jacobus & Gross, 2015). The Sanger sequencing technique on the ABI 3730XL instrument, the DNA products were sequenced (Qi et al., 2024). The obtained sequences were BLASTed using the NCBI GenBank sequence database. Those with 98% or higher similarity to the database were identified at the species level (Schoch et al., 2020).

2.6 Phylogenetic Evaluation

The aim of this study is to distinguish isolates based on their taxonomic similarities at the species level and to reveal their phylogenetic relationships with each other. The bacterial isolates obtained were subjected to the analysis of 16S rRNA gene sequences. Sequence data for each isolate were systematically organized in FASTA format, and the NCBI database was used to identify similar species (Sherry et al., 2001). Only reference sequences obtained from cultured organisms were considered for alignment. Environmental origin sequences, unidentified, or derived from models were excluded from the analysis (Alsanie et al., 2018). To carry out the multitude of sequence comparisons, the sequences acquired during the investigation were aligned with the sequences of the nearest recognized species. The alignment was carried out using MEGA11 software and the ClustalW algorithm for this purpose. After the alignment of the sequences, a phylogenetic tree was created, and assessments were carried out using the Neighbor-Joining (NJ) approach. In the NJ analysis, genetic distances were calculated using the Kimura 2-Parameter (K2P) model (Mahadani et al., 2022). 1000 bootstrap replicates were employed to appraise the reliability of the tree topology. The tree derived from the phylogenetic analysis visually represented the evolutionary relationships of the isolates identified in the study to one another and to the reference species (Lee & Doughty, 1997). This analysis provides significant data for elucidating the phylogenetic

diversity of the microbial communities located in the natural soda water source.

2.7 Statistical Evaluation

The quality parameters of thirty water samples taken from Çerme Village were evaluated using a one-sample t-test. The average values were then compared with the global average values of the World Health Organization (WHO) drinking water standards (Edition, 2011). Diversity was calculated using the Shannon index (H′) based on 16S rRNA sequencing data (Ulucay, 2025).

2.8 Microbial Diversity Analysis (Shannon Index)

In this study, the diversity of the isolated bacterial taxa through the quantitative calculation of the Shannon diversity index (H′). 16S rRNA sequence analysis led to the classification of isolates at the species level and later grouped to compute the relative abundance (p_i) of every bacterial genus in the total samples. The results were used to explain microbial diversity and the dominance of specific taxa in the ecosystem (Schiano-Lomoriello et al., 2024). Calculations were executed utilizing the Python programming language.

3 Results

3.1 Land Features and Water Chemical Findings

Measurements of the spring water revealed a temperature of approximately 20 °C. Based on measurements taken at the central point, the pH value ranged from 7.0 to 7.5. The results of the chemical analyses performed on the physical and chemical water parameters are shown (Table 1). The results obtained when comparing the findings with drinking water standards are also shown in Table 2.

3.2 Morphological Characterization

A total of 12 bacterial isolates were obtained from a soda spring in Çerme Village, Kars Province. Presented here are isolates which were able to grow under varied temperature conditions, hence attesting to their resistance to environmental stress. Morphological and biochemical analyses were carried out for preliminary distinction at species level. Isolates were evaluated according to criteria such as catalase (Fig. 2) and oxidase activity, endospore formation, and cell morphology (Table 3). Each isolate was streaked (Fig. 2). According to Gram staining results, the majority of the isolated bacteria were Gram-positive and morphologically rod-shaped (bacilli)

Table 1 Statistical summary values of basic physical and chemical parameters for 30 different spring water samples collected from Çerme Village. The table shows the mean ($\bar{X}$), standard error (Sx), minimum and maximum values for each parameter

Content	n	$\bar{X}$ (Mean)	Sx (Standard Error)	Minimum	Maximum
pH	5	7.45	0.314	6.62	8.37
Total hardness degree (mS/cm)	5	2.9	0.111	2.61	3.21
Total alkalinity	5	125	5.158	114.2	141.7
Calcium (mg/L)	5	47	2.779	38.76	52.95
Magnesium (mg/L)	5	7.5	0.153	7.5	8.39
Chloride (mg/L)	5	40	0.764	37.93	42.33
Total organic matter (mg/L)	5	2.2	0.062	2.04	2.35
Bicarbonate (mg/L)	5	146	9.471	121.02	176.26
Mangan (mg/L)	5	0.83487	0.06846	0.74912	0.95011
NO_2 (mg/L)	5	0.6	0.035	0.47	0.64
NH_4 (mg/L)	5	0.6	0.021	0.6	0.71
NO_3 (mg/L)	5	48	1.967	43.03	55.15
Fe (mg/L)	5	0.5	0.013	0.49	0.55
Al (mg/L)	5	0.4	0.015	0.35	0.43
Cu (mg/L)	5	3.7	0.175	3.06	4.12
Pb (mg/L)	5	0,0005	5e-05	0.00045	0.00055
S (mg/L)	5	0.6	0.019	0.54	0.64

Table 2 Comparison of the average values of chemical parameters of spring water samples taken from Çerme Village with World Health Organization (WHO) and general drinking water standards. The "Compliance Status" column indicates whether the measured average is within the specified limit range (Edition, 2011)

Parameter	Average Value	Drinking Water Limit	Eligibility Status
pH	7.45	6.5–8.5	Suitable
Total hardness degree (mS/cm)	2.9	0–2.5	Not Suitable
Total alkalinity	125.0	0–200	Suitable
Calcium (mg/L)	47.0	0–100	Suitable
Magnesium (mg/L)	7.5	0–50	Suitable
Chloride (mg/L)	40.0	0–250	Suitable
Total organic matter (mg/L)	2.2	0–3	Suitable
Bicarbonate (mg/L)	146.0	0–500	Suitable
Manganese (mg/L)	0.83487	0–0.05	Not Suitable
NO_2 (mg/L)	0.6	0–0.5	Not Suitable
NH_4 (mg/L)	0.6	0–0.5	Not Suitable
NO_3 (mg/L)	48.0	0–50	Suitable
Fe (mg/L)	0.5	0–0.3	Not Suitable
Al (mg/L)	0.4	0–0.2	Not Suitable
Cu (mg/L)	3.7	0–2	Not Suitable
Pb (mg/L)	0,0005	0–0.01	Suitable
S (mg/L)	0.6	0–0.5	Not Suitable

or spherical (cocci). However, some isolates were observed to be Gram-negative and coccobacilli-like (Figs. 3). Additionally, some isolates were identified as Gram-negative and coccobacillus. When evaluated for endospore formation, only one isolate (*Paenibacillus lautus*) was found to be endospore-forming, while all other strains were endospore-negative. Colony morphology revealed that most isolates formed cream or white colonies, and all isolates showed visible colony development after 24 h of incubation.

3.3 16S rRNA Gene Regions of Isolates

Genomic DNA of the bacteria isolated in this study was obtained following a previously described standard protocol. Subsequently, taxonomically important and evolutionarily conserved regions of the 16S rRNA gene were amplified by PCR using universal primers. The amplified sequences were evaluated against the GenBank database using BLASTn analysis, and the species with the highest similarity were identified. Identification information of the isolates is presented in Table 3. As a result of these analyses, the following bacterial species were identified: *Kurthia gibsonii*, *Staphylococcus sciuri*, *Paenibacillus lautus*, *Aeromonas hydrophila*, *Listeria monocytogenes*, *Staphylococcus saprophyticus*, *Pseudomonas stutzeri*, *Micrococcus luteus*, *Kurthia* sp., *Staphylococcus* sp., *Pseudomonas aeruginosa* and *Micrococcus* sp. All of these species have adapted to pH 7.0 and temperature conditions of 20 °C, and most of them are catalase positive, gram positive and non-spore forming bacteria.

3.4 Phylogenetic

Phylogenetic analyses of the 16S rRNA gene sequences isolated in the study were performed by Neighbor-Joining (NJ) method using MEGA11 software and the evolutionary relationships between species were evaluated. The tree structure was built based on the Kimura 2-parameter model and supported by 1000 bootstrap replicates. The phylogenetic tree study revealed that the isolates of the same genus were grouped together with very good bootstrap support. To illustrate, one group included *Staphylococcus sciuri* (C2), *S. saprophyticus* (C2), and *Staphylococcus* sp. (C6) isolates along with the reference strain *Bacillus subtilis* strain LBY which showed that not only the isolates but also the others of the same branch had similar phylogenetic origins. *Kurthia gibsonii* (C1) and *Kurthia* sp. (C5) isolates were very much near the same branch as *Kurthia gibsonii* strain 1716 with 84% bootstrap support

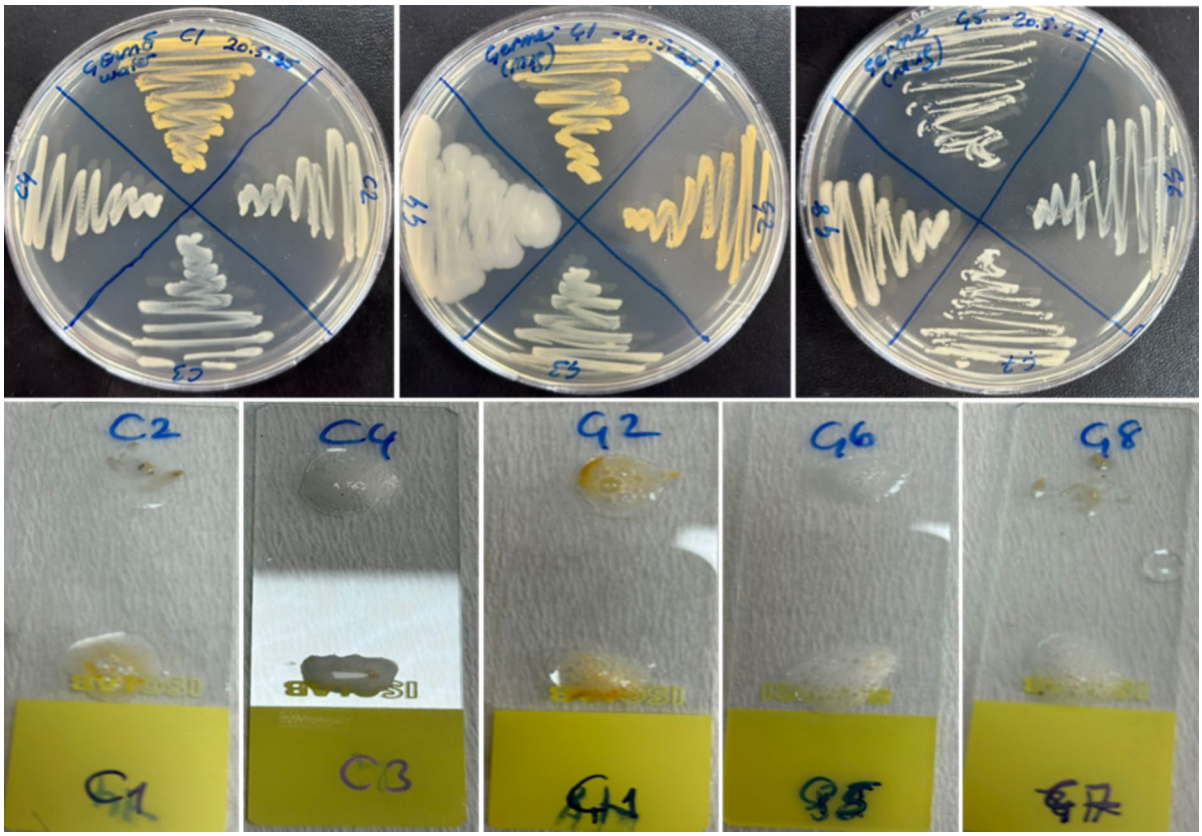


Fig. 2 Colony appearances of pure isolates obtained from the Çerme Village soda spring. Each Petri dish was divided into four sections, and a single-line inoculation technique was used. **A** The first box contains isolates coded C1, C2, C3, and C4; **B** The second box contains isolates coded Ç1, Ç2, Ç3, and Ç4; **C** The third box contains isolates coded Ç5, Ç6, Ç7, and Ç8. The white, cream, and yellow tone differences observed in the colonies indicate the initial morphological differentiation. Catalase test results for isolates. In the catalase test performed by dropping hydrogen peroxide (3% H_2O_2) onto fresh cultures, oxygen bubbles were observed in isolates coded C1, C2, C3, C4, Ç1, Ç2, Ç5, Ç6, and Ç8, consistent with catalase positivity. Foaming was clearly observed in all positive samples

Table 3 Identification and basic phenotypic properties of bacteria isolated from the Çerme Village soda water source at species level

Isolate	Identification	pH	Temperature	Catalase	Oxidase	Endospore	Gram test
C1	*Kurthia gibsonii*	7.0	19–20	+	-	-	+
C2	*Staphylococcus sciuri*	7.0	19–20	+	-	-	+
C3	*Paenibacillus lautus*	7.0	19–20	+	-	+	+
C4	*Aeromonas hydrophila*	7.0	19–20	+	+	-	+
Ç1	*Listeria monocytogenes*	7.0	19–20	+	-	-	-
Ç2	*Staphylococcus saprophyticus*	7.0	19–20	+	-	-	+
Ç3	*Pseudomonas stutzeri*	7.0	19–20	+	+	-	+
Ç4	*Micrococcus luteus*	7.0	19–20	+	+	-	-
Ç5	*Kurthia* sp.	7.0	19–20	+	-	-	+
Ç6	*Staphylococcus* sp.	7.0	19–20	+	-	-	+
Ç7	*Pseudomonas aeruginosa*	7.0	19–20	+	-	-	+
Ç8	*Micrococcus* sp.	7.0	19–20	+	+	-	-

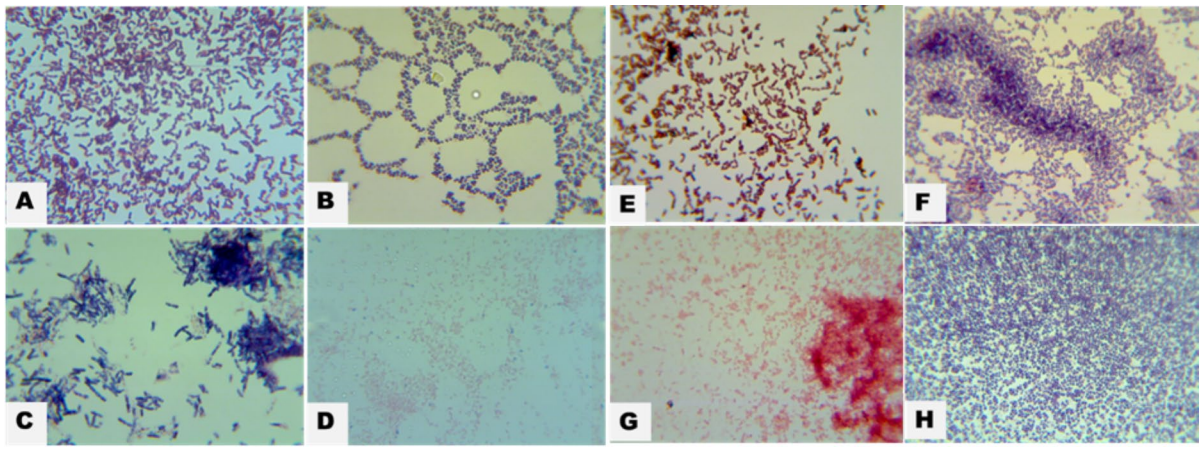


Fig. 3 Gram stain images of some bacterial strains isolated from thermal water samples from Çerme Village. **A** *Kurthia gibsonii*, **B** *Staphylococcus sciuri*, **C** *Paenibacillus lautus*, **D** *Listeria monocytogenes,* **E** *Aeromonas hydrophila*, **F** *Staphylococcus saprophyticus*, **G** *Micrococcus luteus*, and **H** *Pseudomonas stutzeri*

thus denoting these species as genetically very closely related. The *Listeria monocytogenes* isolate (Q1) showed complete similarity with the reference *Listeria monocytogenes* strain NITRR/R1 and these two species were placed in the same phylogenetic branch. Similarly, the *Paenibacillus lautus* (C3) isolate shows high concordance with *Paenibacillus lautus* strain P2 7 in the same branch. The molecular-level determinations of species were supported by this. The Gram-negative isolates consisting of *Pseudomonas stutzeri* (Q3) and *P. aeruginosa* (Q7) clustered together with strong bootstrap support at the same time and at the reference sequences. Similarly, the *Aeromonas hydrophila* (C4) isolate was found to be 100% identical to the reference *A. hydrophila* strain Marseille-P845. *Escherichia* coli strain MK6649 was located further up the tree than all other branches and was successfully used as an outgroup in the analysis (Fig. 4). This suggests that the *E. coli* sequence provides an appropriate reference point for the phylogenetic classification of the isolates in this study. In general, the results of phylogenetic analysis were in agreement with the morphological and biochemical descriptions. These results suggest that the bacteria isolated from the lye water source were correctly classified at the species level and showed high phylogenetic similarity with reference species that thrive in similar environmental conditions.

3.5 Statistical Evaluation

Basic physical and chemical parameters of 30 different samples collected from the natural soda spring in Çerme Village were statistically evaluated. For each parameter, the mean ($\bar{X}$), standard error (Sx), minimum, and maximum values were calculated, and the findings are summarized in Table 1. The findings reflect general trends in the chemical quality of the available water and were also analyzed comparatively with the World Health Organization (WHO), the Turkish drinking water standard (TS 266), and international limit values (Table 2) (Sipahi & Uslu, 2016). On the basis of data collected, the average pH value was measured out to be 7.45 and found to be within the permissible limits in relation to the standard for drinking water (6.5–8.5). In the same manner, the concentrations of calcium, magnesium, chloride, bicarbonate, total alkalinity, and total organic matter did not exceed the allowed limits. Only the total hardness of the water (2.9 mS/cm) surpassed the maximum limit set by Turkish drinking water standards ($\leq$2.5 mS/cm) and was therefore categorized as "not compliant". Moreover, the presence of the contaminants ammonium (NH_4), nitrite (NO_2), and total iron (Fe) indicated that the water quality was unfit for human consumption. Among the elements, manganese (Mn) especially accounted for an average of 0.83487 mg/L (834.87 µg/L), which was nearly 16

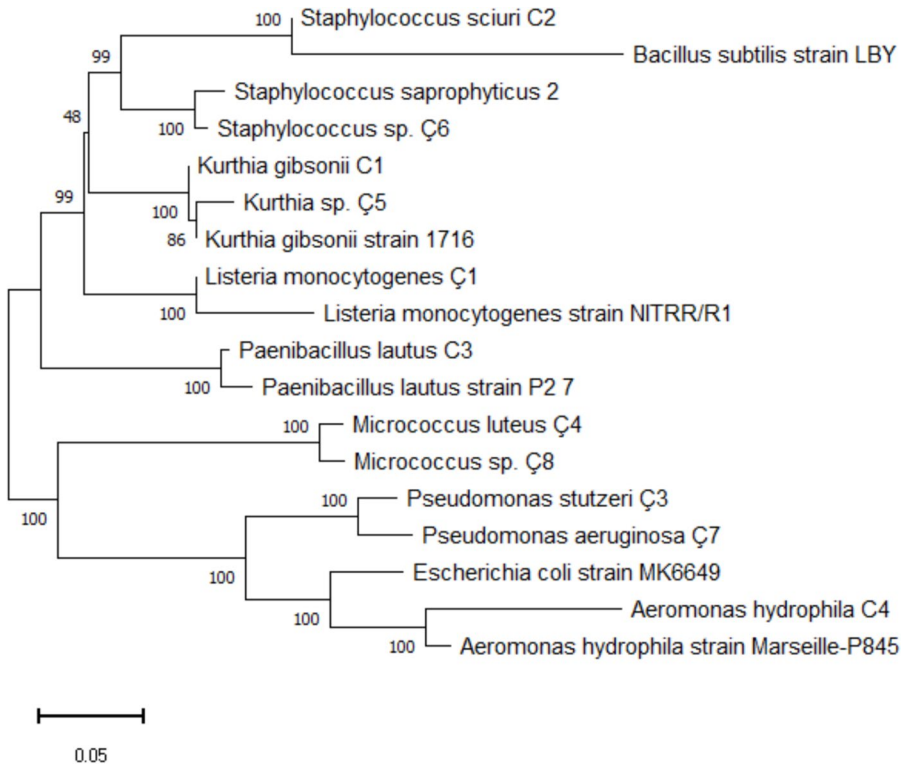


Fig. 4 Phylogenetic tree of 16S rRNA gene sequences of bacterial strains isolated in the study and reference species generated by Neighbor-Joining method. Genetic distances were calculated by Kimura 2-parameter model and reliability values were supported by bootstrap analysis (1000 replicates). *E. coli* was used as an outgroup

times that of the maximum permissible concentration of 0.05 mg/L as set by WHO and TS 266, thus raising a significant concern of environmental and human health. A value of lead (Pb) at 0.0005 mg/L was recorded which, according to the standard, was found to be "suitable" for drinking water, as it was far less than the limit of 0.01 mg/L. The levels of aluminum (Al) and copper (Cu) identified as 0.4 mg/L and 3.7 mg/L, respectively, indicate that they are above the permissible limits for drinking water. On the other hand, the mercury (Hg) concentration was measured at 0.74 µg/L, which is still below the threshold of 1.0 µg/L, but it's still a level that needs to be monitored closely. Moreover, the arsenic (As) concentration of 6.03 µg/L is approaching the margin of safety.

These geochemical inputs from the water's subsurface rock structure and heavy metal accumulation together with possible human influences rise to the surface as major factors in the area of concern for drinking water safety. This scenario will not only be a major area of concern for drinking water safety but also for the microbial species present in the environment, as they will be subject to the very same selection pressures. Bacterial species with metal tolerance can be easily picked out in the exposures, especially with manganese, iron, and arsenic as decisive factors.

3.6 Bacterial Diversity Assessment

Using morphological, biochemical and molecular methods, all together, the 12 strains of bacteria that were isolated in the course of the research were characterized at the species level. The isolates belonged to a total of 7 genera and represented species from these genera. The quantitative assessment of bacterial diversity was done by calculating the Shannon diversity index (H′), which was yielded as $H' = 1.86$. The figure obtained indicates a moderate level of diversity for bacterial communities coming from environmental sources. This level of the Shannon index suggests that certain bacterial genera (such as *Staphylococcus*, *Kurthia*, and *Micrococcus*) are more proficiently adapted to their environment and are represented by multiple isolates, while other genera (such as *Paenibacillus*, *Aeromonas*, and *Listeria*) are less frequently obtained (Table 4). This distribution also points to

Table 4 Distribution of bacterial genera isolated from Çerme Village and their relative frequencies (p_i) used in the calculation of the Shannon diversity index (H′). Data show that 7 different genera were represented across 12 isolates

Bacterial Type	Number of Isolates	Ratio (p_i)
Staphylococcus	3	0.25
Kurthia	2	0.1667
Micrococcus	2	0.1667
Pseudomonas	2	0.1667
Paenibacillus	1	0.0833
Aeromonas	1	0.0833
Listeria	1	0.0833

relationship between the water's chemical composition and the selection pressure on microorganism. Elevated concentrations of elements such as manganese, nitrite, and iron, in particular, may affect the selection of metal-tolerant strains.

4 Discussion

This research intends to assess thoroughly the chemical characteristics of water samples collected from a natural soda spring in Çerme Village, the northeastern part of Turkey, and the varieties of environmental bacteria sourced from these habitats. Incredible discoveries regarding the nature of the water and its microbial inhabitants were uncovered by the analysis results. The chemical examination indicated that the characteristics of water such as pH, calcium, magnesium, chloride, bicarbonate, and total organic matter were congruent with the present drinking water quality guidelines. Nonetheless, it was concluded that the amounts of total hardness, ammonium (NH_4), nitrite (NO_2), iron (Fe), aluminum (Al), copper (Cu), sulfur (S) and particularly manganese (Mn) surpassed the national and international drinking water standards (Edition, 2011). The level of manganese, specifically, was assessed at 0.83487 mg/L, nearly 16 times above the WHO and TS 266 limit of 0.05 mg/L, which means the water source concerned has a considerable heavy metal build-up owing to its geological structure. Besides human health, high manganese levels in drinking water are also a factor for microbial communities' selection pressure in the environment (Piazza et al., 2019). Recent studies have reported that manganese-tolerant bacterial genera such as *Pseudomonas*, *Bacillus*, and *Microbacterium* play important roles in Mn(II) resistance and detoxification processes in natural water systems (Cai et al., 2023; Huang et al., 2020; Li et al., 2024). These findings are consistent with our study and highlight the potential for bioremediation by metal-tolerant bacteria isolated from the Çerme source. The presence of low concentrations of other toxic elements such as arsenic, mercury, and lead indicates that the area is to some extent less impacted by human pollution, and the natural geochemical metals are not insignificant.

As a result of the microbiological analyses, the twelve isolates were identified to species level through morphological, biochemical, and 16S rRNA gene sequencing analyses. Seven different genera of bacteria were obtained, of which *Staphylococcus* spp., *Kurthia* spp., and *Micrococcus* spp. were found to dominate. This implies some bacterial groups thrive and are highly adapted to such environments (Becker et al., 2015; Kloos & Musselwhite, 1975). The Shannon diversity index ($H' = 1.86$) indicates that the Çerme source contains moderate bacterial diversity. The results reveal the effect of environmental and chemical content on the diversity of microorganisms. In particular, high levels of iron, copper, and aluminum, in addition to manganese, may have been effective in the selection of bacterial taxa with metal tolerance; because some genera are known to possess adaptive mechanisms such as metal ion efflux systems, enzymatic detoxification, and cell wall binding. When compared with similar studies in the literature, some bacteria isolated from fountain water samples (e.g. *Pseudomonas* spp., *Paenibacillus* spp. and *Aeromonas* spp.) appear to be among the environmental microorganisms with high metal tolerance (Fakhar et al., 2022; Kanekar & Kanekar, 2022; Vélez et al., 2021). Both *Listeria monocytogenes* and *Staphylococcus saprophyticus* are potential pathogens that, along with the other pathogens, signal the public health risks of uncontrolled use of this water source. The study findings, along with the fact these species were previously isolated from iron, manganese, and arsenic rich environments, highlight the ability of the isolated strains to tolerate a variety of environmental stresses (Sousa et al., 2017; Troller, 1986). Individuals working with animals in the open areas, as well as those suffering from compromised immunity, are greatly affected by the presence of these bacteria. In addition, the phylogenetic analysis results matched

with the other investigations, and the isolates closely matched the reference species. This adds more credibility to the molecular species identifications and shows that the isolates were correctly delimited from the taxonomic perspective. Moreover, the fact that species of the same genus cluster together on similar branches with high bootstrap values in the phylogenetic tree supports their genetic relatedness as well as similarities in their adaptation to the environment.

This study presents one of the first detailed chemical and microbiological analyses performed on a natural spring water that is traditionally consumed but not sufficiently documented with scientific data. It was decided that those natural resources, particularly those situated in remote areas and utilized for human or livestock water supply, should be subject to frequent monitoring because of the risks which they may pose. The data derived from the study also give significant hints regarding the relationship between microbial diversity and environmental chemical factors, since it has been demonstrated that "the fittest" are the ones that thrive in heavy metal polluted areas. The discoveries have been a source of significant information not only for local authorities in policy making but also for future research in biotechnology and environment. The primary premise of this research is the assumption that high manganese concentration at the Çerme spring is a factor that influences the living conditions of the microbial communities in the environment. In this context, the study intends to analyze the spring water chemically, recognize the diverse bacteria, and correlate the metal content with the microbial diversity.

5 Conclusions

This research paper is among the pioneering studies that have been able to carry out a complete and thorough evaluation both chemically with the help of several instruments and microbiologically of a natural soda water source that has been traditionally used for drinking and has been neglected in terms of scientific evaluation. The outcome demonstrated that some of the chemical parameters of the water (namely manganese, iron, nitrite, ammonium) were far above the drinking water standards by a significant margin. The matter not only has negative implications for human health but also gives an indication that the microbial diversity of the environment is determined to some extent by the aforementioned chemical conditions. In the microbiological analysis, the identification of 12 isolates, mainly consisting of Gram-positive and resistant to environmental stress conditions, was made, and the Shannon diversity index value of ($H' = 1.86$) showed a moderate level of diversity. These findings emphasized the fact that regular monitoring of such natural water sources in rural areas, which are used by both animals and humans, is necessary. This research served as an excellent reference for future work tracing the presence of metal-tolerant environmental bacteria.

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References

Alsanie, W. F., Felemban, E. M., Farid, M. A., Hassan, M. M., Sabry, A., & Gaber, A. (2018). Molecular identification and phylogenetic analysis of multidrug-resistant bacteria

using 16S rDNA sequencing. *Journal of Pure and Applied Microbiology, 12*, 489–496.

Apostolaki, S., Akinsete, E., Koundouri, P., & Samartzis, P. (2020). Freshwater: The importance of freshwater for providing ecosystem services. In: *Encyclopedia of the World's biomes* (pp. 71–79).

Becker, K., Skov, R. L., & von Eiff, C. (2015). Staphylococcus, Micrococcus, and other catalase-positive cocci. *Manual of clinical microbiology* (pp. 354–382).

Borzenko, S. V., Zamana, L. V., & Usmanova, L. I. (2018). Basic formation mechanisms of Lake Doroninskoye soda water, East Siberia, Russia. *Acta Geochimica, 37*, 546–558.

Cai, Y., Yang, K., Qiu, C., Bi, Y., Tian, B., & Bi, X. (2023). A review of manganese-oxidizing bacteria (MnOB): Applications, future concerns, and challenges. *International Journal of Environmental Research and Public Health, 20*, Article 1272.

Edition, F. (2011). Guidelines for drinking-water quality. *WHO Chronicle, 38*, 104–108.

El Ahmadi, K., Haboubi, K., El Allaoui, H., El Hammoudani, Y., Bouhrim, M., Eto, B., Shahat, A. A., & Herqash, R. N. (2025). Isolation and preliminary screening of lactic acid bacteria for antimicrobial potential from raw milk. *Frontiers in Microbiology, 16*, 1565016.

Fakhar, A., Gul, B., Gurmani, A. R., Khan, S. M., Ali, S., Sultan, T., Chaudhary, H. J., Rafique, M., & Rizwan, M. (2022). Heavy metal remediation and resistance mechanism of *Aeromonas*, *Bacillus*, and *Pseudomonas*: A review. *Critical Reviews in Environmental Science and Technology, 52*, 1868–1914.

Gadd, G. M. (2010). Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology, 156*, 609–643.

https://gisgeography.com/turkey-map/#Satellite-Map: 2025, 'Türkiye geological map', https://gisgeography.com/turkey-map/#Satellite-Map

Huang, H., Zhao, Y., Xu, Z., Ding, Y., Zhou, X., & Dong, M. (2020). A high Mn (II)-tolerance strain, *Bacillus thuringiensis* HM7, isolated from manganese ore and its biosorption characteristics. *PeerJ, 8*, Article e8589.

Jacobus, A. P., & Gross, J. (2015). Optimal cloning of PCR fragments by homologous recombination in *Escherichia coli*. *PLoS ONE, 10*, Article e0119221.

Kanekar, P. P., & Kanekar, S. P. (2022). *Metallophilic, metal-resistant, and metal-tolerant microorganisms* (pp. 187–213). Springer.

Kloos, W. E., & Musselwhite, M. S. (1975). Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Applied Microbiology, 30*, 381–395.

Laanbroek, H. J. (1990). Bacterial cycling of minerals that affect plant growth in waterlogged soils: A review. *Aquatic Botany, 38*, 109–125.

Lagier, J.-C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. *Clinical Microbiology Reviews, 28*, 208–236.

Lee, M. S. Y., & Doughty, P. (1997). The relationship between evolutionary theory and phylogenetic analysis. *Biological Reviews, 72*, 471–495.

Li, Z., Wang, J., Deng, R., Azuan, L., Chen, M., He, X., & Yue, Z. (2024). Mechanisms of manganese-tolerant *Bacillus brevis* MM2 mediated oxytetracycline biodegradation process. *Environmental Pollution, 363*, Article 125060.

Mahadani, A. K., Awasthi, S., Sanyal, G., Bhattacharjee, P., & Pippal, S. (2022). Indel-K2P: A modified Kimura 2 parameters (K2P) model to incorporate insertion and deletion (Indel) information in phylogenetic analysis. *Cyber-Physical Systems, 8*, 32–44.

Mangoma, N., Zhou, N., & Ncube, T. (2024). Metagenomic insights into the microbial community of the Buhera soda pans, Zimbabwe. *BMC Microbiology, 24*, 510.

Mhammedsharif, R. M., & Kolo, K. Y. (2023). A case study of environmental pollution by pathogenic bacteria and metal (oid) s at Soran Landfill Site, Erbil, Iraqi Kurdistan Region. *Environmental Monitoring and Assessment, 195*, 811.

Mijnendonckx, K., Smolders, C., Bartak, D., Le Duc, T., Morales-Hidalgo, M., Povedano-Priego, C., Jroundi, F., Merroun, M. L., Leys, N., & Cerna, K. (2024). Comparing the effectiveness of different DNA extraction methods in MX-80 bentonite. *Environmental Microbiology Reports, 16*, Article e70047.

Nnaji, N. D., Anyanwu, C. U., Miri, T., & Onyeaka, H. (2024). Mechanisms of heavy metal tolerance in bacteria: A review. *Sustainability, 16*, Article 11124.

Oluseyi Osunmakinde, C., Selvarajan, R., Mamba, B. B., & Msagati, T. A. M. (2019). Profiling bacterial diversity and potential pathogens in wastewater treatment plants using high-throughput sequencing analysis. *Microorganisms, 7*, 506.

Özgür, N., & Büyükşahin, S. (2019, November). Hydrogeological Conceptual Modelling of Geothermal Waters in Urganlı, Western Anatolia, Turkey. In *Conference of the Arabian Journal of Geosciences* (pp. 593–595).

Piazza, A., Ciancio Casalini, L., Pacini, V. A., Sanguinetti, G., Ottado, J., & Gottig, N. (2019). Environmental bacteria involved in manganese (II) oxidation and removal from groundwater. *Frontiers in Microbiology, 10*, 119.

Qi, G., Hao, L., Gan, Y., Xin, T., Lou, Q., Xu, W., & Song, J. (2024). Identification of closely related species in *Aspergillus* through analysis of whole-genome. *Frontiers in Microbiology, 15*, 1323572.

Ramljak, A., Žižek, M., Huđ, A., Palijan, G., Lučić, M., & Petrić, I. (2025). Resisting the toxic tide: Multi-metal resistance of bacteria originating from contaminated Šibenik Bay sediments. *Microorganisms, 13*, 2326.

Roy, R., Samanta, S., Pandit, S., Naaz, T., Banerjee, S., Rawat, J. M., Chaubey, K. K., & Saha, R. P. (2024). An overview of bacteria-mediated heavy metal bioremediation strategies. *Applied Biochemistry and Biotechnology, 196*, 1712–1751.

Schiano-Lomoriello, D., Abicca, I., Contento, L., Gabrielli, F., Alfonsi, C., Di Pietro, F., Papa, F. T., Ballesteros-Sánchez, A., Sánchez-González, J.-M., & Rocha-De-Lossada, C. (2024). Infectious keratitis: Characterization of microbial diversity through species richness and shannon diversity index. *Biomolecules, 14*, 389.

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., McVeigh, R.,

O'Neill, K., & Robbertse, B. (2020). Ncbi taxonomy: A comprehensive update on curation, resources and tools. *Database, 2020*, Article baaa062.

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: The NCBI database of genetic variation. *Nucleic Acids Research, 29*, 308–311.

Sipahi, F., & Uslu, S. (2016). Investigation of the quality and physical-geochemical characteristics of the drinking water in Gümüşhane (Turkey) city central. *Arabian Journal of Geosciences, 9*, 600.

Sousa, A. M., Machado, I., Nicolau, A., & Pereira, M. O. (2013). Improvements on colony morphology identification towards bacterial profiling. *Journal of Microbiological Methods, 95*, 327–335.

Sousa, VSd., da-Silva, APd. S., Sorenson, L., Paschoal, R. P., Rabello, R. F., Campana, E. H., Pinheiro, M. S., Santos, L. OFd., Martins, N., & Botelho, A. C. N. (2017). *Staphylococcus saprophyticus* recovered from humans, food, and recreational waters in Rio de Janeiro, Brazil. *International Journal of Microbiology, 2017*, Article 4287547.

Sukanya, S., & Joseph, S. (2024). *Alternative water resources in rural areas: Smart solutions for a sustainable future* (pp. 107–116). Elsevier.

Sun, Y., Wu, Z., Lan, J., Liu, Y., Du, Y., Ye, H., & Du, D. (2025). Effect of sulfate-reducing bacteria (SRB) and dissimilatory iron-reducing bacteria (DIRB) coexistence on the transport and transformation of arsenic in sediments. *Water Research, 270*, Article 122834.

Troller, J. A. (1986). Water relations of foodborne bacterial pathogens-An updated review. *Journal of Food Protection, 49*, 656–670.

Ulucay, O. (2025). 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*. https://doi.org/10.1016/j.jece.2025.118059

Ulucay, O., Gormez, A., & Ozic, C. (2022a). For biotechnological applications: Purification and characterization of recombinant and nanoconjugated xylanase enzyme from thermophilic *Bacillus subtilis*. *Biocatalysis and Agricultural Biotechnology, 44*, Article 102478.

Ulucay, O., Gormez, A., & Ozic, C. (2022b). Identification, characterization and hydrolase producing performance of thermophilic bacteria: geothermal hot springs in the Eastern and Southeastern Anatolia Regions of Turkey. *Antonie van Leeuwenhoek*, *115*(2), 253-270.

Vélez, J. M. B., Martínez, J. G., Ospina, J. T., & Agudelo, S. O. (2021). Bioremediation potential of *Pseudomonas* genus isolates from residual water, capable of tolerating lead through mechanisms of exopolysaccharide production and biosorption. *Biotechnology Reports, 32*, Article e00685.

Zhang, Q., Achal, V., Xiang, W. N., & Wang, D. (2014). Identification of heavy metal resistant bacteria isolated from Yangtze River, China. *International Journal of Agriculture and Biology, 16*, 619–623.

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