Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 35, issue 3, 2025, pp. 95 - 105

MICROBIAL COMMUNITY COMPOSITION AND DIVERSITY IN THE HADJER EL-MELH HYPERSALINE ECOSYSTEM (DJELFA, ALGERIA)

Ahmed BELADEL[1], Saad BOUTAIBA[1], Brahim BELADEL[2], Fahd ARBAOUI[3], Aissa BENHAMIDA[4], Yazid KHALED KHODJA[1], Mostapha BACHIR-BEY[5*], Mohammed Mebarek BIA[6]

[1]Laboratory of Exploration and Valorization of Steppe Ecosystems, Faculty of Natural Sciences and Life, University of Djelfa, PO Box 3117, Djelfa 17000, Algeria
[2]Department of Physics, Faculty of Exact Sciences and Informatics, University of Djelfa, PO Box 3117, Djelfa 17000, Algeria
[3]Nuclear Research Centre of Birine (CRNB), PB 180, 17200, Ain Oussera, Djelfa, Algeria
[4]Department of Biology, Faculty of Natural Sciences and Life, University of Chahid Hama Lakhdar, 39000 El-Oued, Algeria
[5]University of Bejaia, Faculty of Natural and Life Sciences, Laboratory of Applied Biochemistry, 06000 Bejaia, Algeria
[6]Department of Parasitology, College of Medicine, Chungbuk National University, Cheongju, South Korea

Abstract: Hadjer El-Melh ("Salt Rock"), located 24 km north of Djelfa, Algeria, represents a major but understudied athalassohaline hypersaline ecosystem. This study integrates physicochemical analyses with microbial community profiling to explore its microbial diversity. Water samples collected from six sites displayed near-neutral pH values (6.7–7.0) and extremely high salinity (24–27% w/v), with sodium and chloride ions being predominant. Microbial analysis using 16S rDNA sequencing revealed a rich archaeal and bacterial community, with archaea primarily represented by Halobacterota, Nanohaloarchaeota, and Nanoarchaeota, and bacteria mainly by Bacteroidota, Proteobacteria, and Firmicutes. High Operational Taxonomic Unit (OTU) richness and Shannon diversity indices indicated a well-structured microbial community, particularly at sites S5 and S6. These findings provide new insights into hypersaline microbial ecology and serve as a foundation for future environmental and biotechnological research.

Keywords: hypersaline environment, microbial diversity, archaea, bacteria, salt rock, Hadjer El-Melh.

INTRODUCTION

Hypersaline ecosystems are among the most widely distributed extreme habitats worldwide, including deep-sea basins, coastal lagoons, solar salterns, and inland salt lakes with salinities exceeding that of seawater (Wang *et al.*, 2024; Canganella and Wiegel, 2011). In addition to elevated salinity, these environments are often characterized by high alkalinity, intense solar radiation, large temperature fluctuations, and low oxygen availability, making them particularly challenging for biological life (Fernández *et al.*, 2014). Based on their origin, hypersaline systems are generally classified as thalassohaline or athalassohaline ecosystems. Thalassohaline environments originate from marine waters and retain an ionic composition similar to seawater, typically dominated by sodium and chloride ions, as observed in coastal salterns and marine basins (Fernández *et al.*, 2014). In contrast, athalassohaline ecosystems are non-marine in origin, and their chemical composition is primarily shaped by evaporation processes and mineral dissolution, resulting in variable ionic dominance that may include sodium, magnesium, potassium, or carbonate ions depending on local geology and climatic conditions (Youssef *et al.*, 2012; Martínez *et al.*, 2022).

Despite these extreme physicochemical constraints, hypersaline environments host diverse and highly specialized microbial communities, dominated by halophilic archaea, halotolerant and halophilic bacteria, and, to a lesser extent, eukaryotic microorganisms such as algae (Boutaiba *et al.*, 2011; Naghoni *et al.*, 2017; Poli *et al.*, 2011). The persistence of life in such habitats is enabled by a range of physiological and molecular adaptations, including efficient osmoregulatory strategies, synthesis or uptake of compatible solutes, specialized membrane structures, and genetic adaptations that ensure cellular stability under osmotic stress (Ma *et al.*, 2010; Edbeib *et al.*, 2016). These extremophiles play essential ecological roles in biogeochemical cycles, contribute to the formation of microbial mats and evaporitic mineral structures, and represent a valuable reservoir of biomolecules with potential applications in industrial and environmental biotechnology (Van den Burg, 2003; Pikuta *et al.*, 2007; Madsen, 2011; Cowan *et al.*, 2024).

Algeria hosts a wide range of natural extreme environments, particularly hypersaline lakes, Sebkhas, and salt flats distributed across arid and semi-arid regions, making it an important area for the study of halophilic microbial diversity (Boutaiba *et al.*, 2011; Sahli *et al.*, 2020; Beddal *et al.*, 2022). Previous studies in Algeria have mainly addressed halophilic archaea and bacteria from other hypersaline ecosystems (Menasria *et al.*, 2018, 2019; Sahli *et al.*, 2020; Beddal *et al.*, 2022). However, many inland hypersaline systems remain poorly explored, and the relationships between local physicochemical conditions, geomorphological heterogeneity, and microbial community structure are still insufficiently understood. The present study therefore aims to investigate the microbial diversity and physicochemical properties of

***Correspondence**: Mostapha Bachir-Bey, University of Bejaia, Faculty of Natural and Life Sciences, Laboratory of Applied Biochemistry, 06000 Bejaia, Algeria, email: mostapha.bachirbey@univ-bejaia.dz*

Hadjer El-Melh to better understand the environmental factors shaping microbial communities.

Hadjer El-Melh is a large natural rock salt formation situated in the Djelfa region of central Algeria, approximately 24 km north of Djelfa city. This athalassohaline hypersaline ecosystem is characterized by active salt precipitation, extensive white salt crusts, polygonal desiccation patterns, laminated fine-grained evaporites, and locally steeply dipping bedded salt deposits, reflecting complex depositional and post-depositional processes. These features generate pronounced spatial heterogeneity and a diversity of microhabitats, which are likely to influence microbial community assembly and distribution. Despite its geological and ecological significance, Hadjer El-Melh has received little attention from a microbiological perspective.

In this context, the present study aims to investigate the microbial community composition and diversity of the Hadjer El-Melh hypersaline ecosystem, Djelfa, Algeria, in conjunction with an assessment of its physicochemical characteristics. By exploring the relationships between environmental factors and microbial assemblages, this work seeks to improve our understanding of microbial adaptation and community structuring in inland hypersaline environments and to contribute new data on the microbial biodiversity of poorly studied hypersaline ecosystems in North Africa.

MATERIALS AND METHODS

Description of sampling site

Hadjer El-Melh is a large natural rock salt formation located 24 km north of Djelfa city and approximately 275 km south of the Mediterranean Sea (Rabehi *et al.*, 2023), at coordinates 34°77'39.04″ N and 3°17'37.89″ E, with an altitude of 1089 m (Fig. 1).

The Djelfa region has a continental Mediterranean climate, characterized by hot summers, cold winters, and limited precipitation (Bouteldjaoui *et al.*, 2019). The site exhibits active salt precipitation along shallow channels and in surface depressions, forming extensive white salt crusts, polygonal desiccation textures, and laminated, fine-grained evaporitic host rocks. Locally, steeply dipping bedded evaporites are exposed, reflecting complex depositional and post-depositional processes. These geomorphological features create spatially heterogeneous microhabitats, which likely contribute to variability in microbial community structure (Taher, 2014; McGonigle *et al.*, 2021; Martínez-Alvarez *et al.*, 2022).

Sample collection

Sampling occurred during peak spring in last March. Sterile bottles, gloves, and analytical instruments (pH meter, conductometer, thermometer) were prepared beforehand. Water samples were collected in 1.5 L inert bottles to prevent chemical interactions, following established field protocols to ensure quality assurance and control (Zhang and Zhang, 2014). The selection of sampling sites was guided by heterogeneity in key physicochemical properties of the water, namely their visual appearance (color), olfactory characteristics (odor), and hydrological dynamics (flow rate). A total of six hypersaline spring water samples were collected aseptically and coded as S1, S2, S3, S5, S6, and S7.

Analyses of water samples

pH, conductivity, and temperature were measured in triplicate before transporting samples to the laboratory. Elemental concentrations were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Samples were filtered through 0.45 µm membranes, and the ICP-OES was calibrated according to the manufacturer's instructions.

DNA extraction, amplification, and bioinformatics analyses

Water samples were stored at 4 °C in the dark prior to processing. Samples were filtered through 0.2 µm pore-size membranes to collect microbial biomass. Genomic DNA was extracted using a modified CTAB bead-beating method as described by Griffiths *et al.* (2000). The V3–V4 region of the 16S rRNA gene was amplified by PCR using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions were performed using a high-fidelity DNA polymerase under the following conditions: initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, with a final extension at 72 °C for 7 min. Indexed adapters were added to the primers for multiplex sequencing. PCR products were mixed with 2× loading buffer and visualized by 1.8% agarose gel electrophoresis. Amplicons showing a clear band at approximately 450 bp were selected and pooled in equimolar concentrations.

The pooled PCR products were purified using a GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing libraries were prepared using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) according to the manufacturer's instructions. Library quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and concentrations were determined using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Finally, paired-end sequencing (2 × 250 bp) was performed on an Illumina platform (Illumina Inc., San Diego, CA, USA) at Macrogen (South Korea).

(a). Djelfa department (Algeria)

(b). Google earth position of Hadjr El-Melh

(c). Studied site location of Hadjer El-Melh, Djelfa, Algeria (Google Earth).

(d). Field view: Rhythmic evaporite layering with a possible soft-sediment deformation (*Source: Authors).*

(e). Field view (ex. S1): salt-crust-lined channels, brine pools, bedded evaporites (*Source: Authors).*

(f). Field view: salt-crust-lined channels, brine pools, bedded evaporites (*Source: Authors).*

Fig. 1. Geographic location and geomorphological characteristics of the Hadjer El-Melh hypersaline site (Djelfa, Algeria). (a) The geographic position of Djelfa within Algeria, (b) Location of studied site at the regional scale in Djelfa department, (c) Satellite imagery of the Hadjer El-Melh salt depression, and (d–f) representative field photographs illustrating rhythmic evaporite layering, salt-crust-lined channels, brine pools, and bedded evaporitic deposits.

Bioinformatics analysis

Raw reads were quality-checked with FastQC v0.11.3, trimmed for adapters and low-quality bases using Trimmomatic v0.36, and merged in Geneious 9.1.8. ASVs were identified with DADA2 in QIIME2 (Callahan *et al.*, 2016; Bolyen *et al.*, 2019). Taxonomic assignment was performed using a Naive Bayes classifier against the SILVA and UNITE databases (Bokulich *et al.*, 2018). Complementary OTU-based classification was conducted with SILVAngs v1.7.01. Alpha diversity and species richness were calculated using QIIME2 scripts, including Shannon, Simpson, Chao1, and ACE indices, to assess microbial community diversity and sampling completeness.

Raw and processed sequencing data were deposited in the NCBI database under BioProject PRJNA1209385 and BioSample SAMN46220246.

Alpha diversity and SHE analysis

Alpha diversity of the microbial community was assessed using multiple complementary metrics. Shannon and Simpson indices were calculated to evaluate community diversity and dominance (Shannon, 1948; Simpson, 1949) while Chao1 and ACE richness estimators were used to infer species richness and the contribution of rare taxa (Chao *et al.*, 2021). SHE analysis was performed to examine the relationship among species richness (S) Shannon diversity (H), and evenness (E), providing insight into community structure (Hayk and Buzas, 1997). Sampling completeness was assessed using Good's coverage (u) based on the proportion of singleton OTUs relative to the total number of sequences per sample, and rarefaction curves, ensuring that sequencing depth was sufficient to capture the majority of taxa in each sample (McMurdie and Holmes, 2013)

Beta diversity analysis

Beta diversity was assessed to describe differences in microbial community composition among the six samples. Whittaker's beta diversity index (β_W) was calculated based on taxa richness to estimate overall species turnover among samples. In addition, Bray-Curtis dissimilarity was computed from relative abundance data to evaluate abundance-based community differences. The Bray-Curtis matrix was visualized using Principal Coordinates Analysis (PCoA). All beta-diversity analyses were conducted for descriptive purposes.

RESULTS

Physicochemical properties

The mineral assemblages and textures indicate deposition in a restricted evaporitic basin (sabkha/salina or continental saline lake) (Sheikheh *et al.*, 2025; Warren, 2016). Hadjer El-Melh displays typical hypersaline features with salinity between 24–27%, close to globally recognized hypersaline lakes such as Lake Assal (27.7%) and the Dead Sea (34%). pH was near-neutral (6.7–7.0), similar to Algerian lakes Sidi Ameur and Himalatt, but lower than alkaline systems like Wadi Natrun (pH 11) or Lake El Golea (pH 9.0). Sodium and chloride were dominant ions, with Na^+ concentrations ranging from 4–134 g/L and Cl^- from 177–283 g/L (Table 1). In comparison, Algerian hypersaline lakes exhibit lower divalent cation concentrations and moderate salinity, while marine environments have much lower salinity and ionic concentrations. These conditions indicate strong selective pressure on microbial communities in Hadjer El-Melh.

Table 1.

Physicochemical characteristics of selected hypersaline and marine ecosystems

Ecosystem	pH	Na^+ (g/L)	K^+ (g/L)	Mg^{2+} (g/L)	Ca^{2+} (g/L)	Cl^- (g/L)	Salinity (%)	Reference
Hypersaline ecosystems								
Hadjer El-Melh (Algeria)	6.7–7.0	4–134	0.09–8.25	0.41–1.08	0.56–2.53	177–283	24–27	This study
Great Salt Lake (USA)	7.7	105	6.7	11.1	0.3	181	33.3	Bunce *et al.*, 2025
Lake Assal (Djibouti)	n.d.	77.8	5.4	8.0	14.6	164	27.7	Gavrieli, 1997
Dead Sea (Palestine)	7.8	40.1	7.6	44.0	78.2	225	34.0	Khlaifat *et al.*, 2010
Wadi Natrun (Egypt)	11.0	142	2.3	< LOD	< LOD	155	39.4	Mahmoud *et al.*, 2024
El Goléa Lake (Algeria)	9.0	107	n.d.	0.3	0.4	198	29.6	Hacène *et al.*, 2004
Sidi Ameur Lake (Algeria)	7.4	67.1	0.17	3.0	0.51	111	20.0	Boutaiba *et al.*, 2011
Himalatt Lake (Algeria)	7.2	24.5	0.12	1.6	0.22	63.8	11.7	Boutaiba *et al.*, 2011
Marine environments								
Aral Sea	8.2	2.2	0.08	0.55	0.51	3.47	1.02	Imhoff *et al.*, 1986
Caspian Sea	8.3	3.18	0.09	0.73	0.34	5.33	1.28	Oren, 2002
Atlantic Ocean	8.5	10.6	0.38	1.29	0.42	19.2	3.48	Post, 1981

n.d., not determined; LOD, limit of detection; The notation **<** LOD denotes ion concentrations below the detection threshold of the analytical technique used.

Microbial community composition

High-throughput sequencing recovered 1,602 OTUs (782 archaeal, 820 bacterial), with archaeal richness highest in S6 (499 OTUs) and lowest in S2 (123 OTUs), while bacterial richness peaked in S5 (539 OTUs) and was minimal in S2 (60 OTUs). Community composition was strongly archaeal-dominated, with Halobacterota representing ~41–97% of reads, reaching ~97% in S3. Nanohaloarchaeota and Nanoarchaeota contributed substantially in S5 and S6 (up to ~28% and ~24%, respectively), yielding more balanced archaeal assemblages at these sites. Bacterial communities were more diverse and mainly composed of Bacteroidota (8–23%) and Proteobacteria (1–13%), with Bacteroidota enriched in S7 (~23%) and Proteobacteria in S5 (~13%). Firmicutes and Actinobacteriota remained minor (<4%). Overall, S5 and S6 exhibited mixed archaeal–bacterial profiles, whereas S3 showed

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 35, issue 3, 2025, pp. 95 – 105

extreme archaeal dominance, reflecting strong salinity filtering.

At finer taxonomic resolution, Halomicrobiaceae and Nanosalinaceae dominated archaeal assemblages, while Balneolaceae and Moraxellaceae were consistently detected among bacteria. Genera such as Halorientalis, Halomicrobium, Halonotius, and Halorubrum showed site-specific distributions, indicating niche specialization driven by local environmental gradients (Fig. 2).

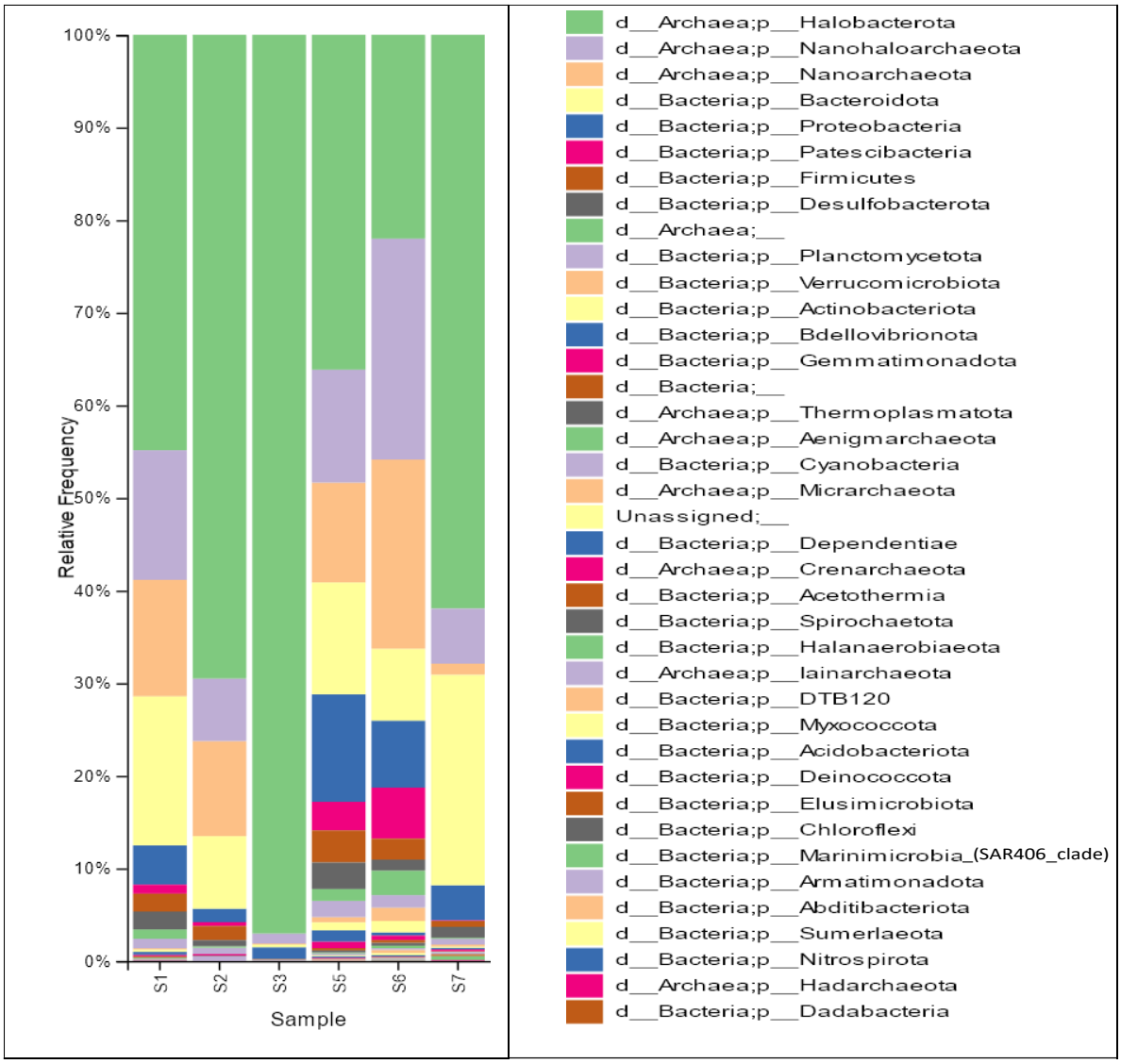

Fig. 2. Phylum-level taxonomic composition of microbial communities across hypersaline samples.

Diversity analysis

Alpha diversity analysis revealed pronounced differences among samples. Shannon diversity (H') was markedly reduced at S3 (H' = 1.46), indicating strong community dominance under extreme hypersaline conditions, whereas samples S5 and S6 exhibited the highest diversity (H' > 5.5). Simpson's index (1–D) confirmed this pattern, with substantially lower values at S3 reflecting reduced diversity and pronounced dominance by a limited number of taxa.

Evenness patterns were further supported by the exp(H)/S index and dominance metrics, with S3 exhibiting extremely low evenness and elevated dominance, consistent with strong taxonomic imbalance. Richness estimators (Chao-1 and ACE) closely matched observed richness, suggesting adequate sampling effort across all sites. Rarefaction curves (Fig. 3) corroborated these results, with all samples approaching asymptotes, indicating sufficient sequencing depth to capture most taxa.

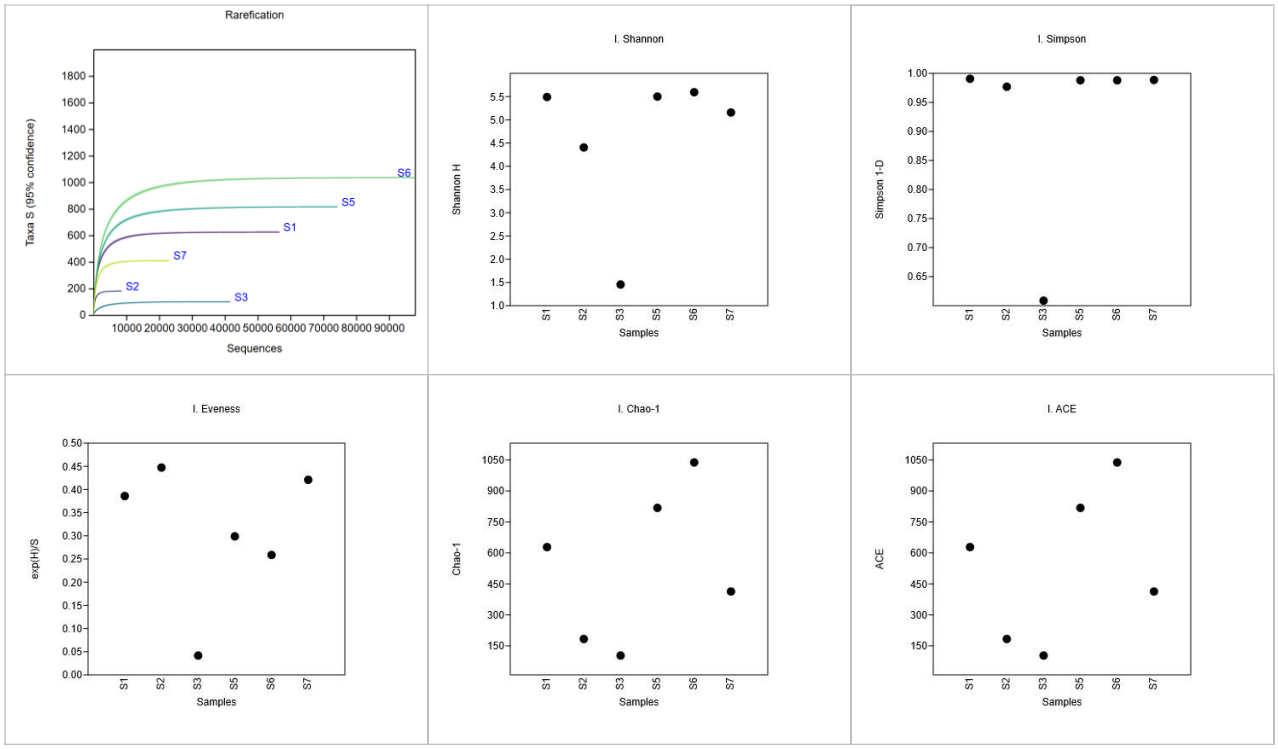

Fig. 3. Alpha diversity indices and rarefaction curves of hypersaline microbial communities.

SHE analysis revealed that variations in Shannon diversity were primarily driven by changes in evenness rather than species richness. While ln S and ln N increased progressively across samples, a marked decrease in Shannon diversity was observed at sample 3, accompanied by a strong reduction in evenness (ln E = −2.03). These results indicate that microbial diversity patterns in hypersaline environments are mainly governed by dominance effects associated with extreme environmental stress (Fig. 4). Sequencing depth was sufficient across all samples, with Good's coverage values exceeding 0.999, indicating that more than 99.9% of the estimated community diversity was captured. Total read counts ranged from 8648 to 98042 sequences per sample, with observed OTU richness (S_obs) varying from 183 to 1038 (Fig. 5). Stable H and low ln E values indicate dominance-driven microbial communities, while increasing ln S reflects OTU accumulation.

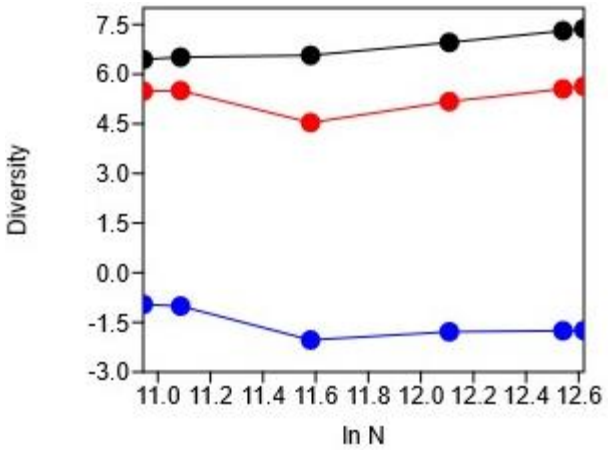

Fig. 4. SHE analysis illustrating changes in species richness (ln S) (black), Shannon diversity (H) (red), and evenness (ln E) (blue) with sequencing depth.

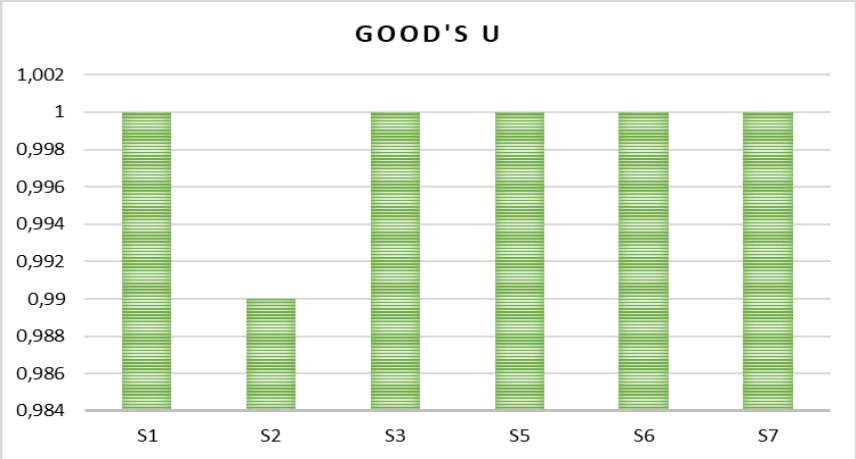

Fig. 5. Good's coverage (Good's *u*) estimates across samples.

Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity of relative abundance data revealed clear compositional differences among the six hypersaline samples (Fig. 6). Consistent with the overall level of community turnover indicated by Whittaker's beta diversity (βw = 2.02), the first coordinate (PCo1) separated sample S3 distinctly from all other samples, indicating a markedly different microbial community composition dominated by specific taxa. In contrast, samples S1, S2, and S7 clustered closely along the negative side of PCo1, suggesting similar community structures among these sites. Samples S5 and S6 were separated primarily along the second coordinate (PCo2), reflecting secondary compositional variation, potentially related to differences in the relative abundance of dominant and rare halophilic taxa. Together, these patterns illustrate distinct differences in microbial community composition among the samples.

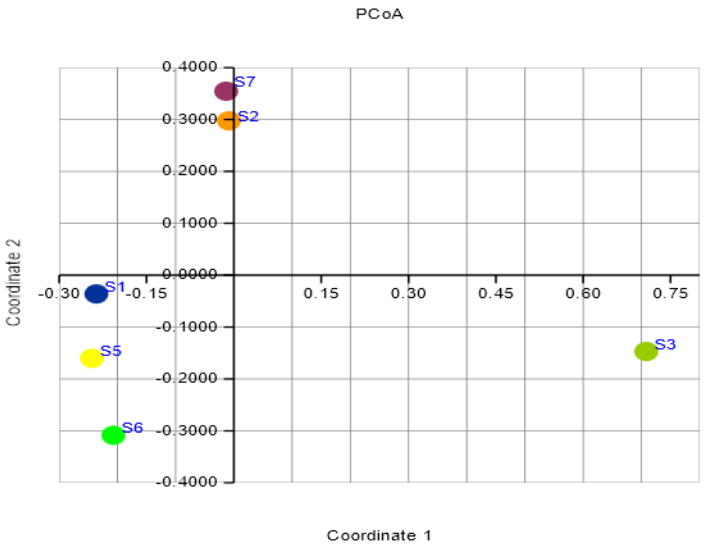

Fig. 6. Principal Coordinates Analysis (PCoA) of microbial community composition based on Bray–Curtis dissimilarity calculated from relative abundance data. Each point represents hypersaline samples. Spatial separation among samples reflects differences in community composition driven by shifts in dominant and rare taxa. Samples clustering together indicate similar microbial assemblages, while distant samples indicate strong compositional divergence.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that the dominant OTUs recovered from the hypersaline samples clustered within well-defined archaeal and bacterial lineages (Fig. 7). Most OTUs affiliated with halophilic archaea, including members of the genera *Halorubrum*, *Halomicrobium*, *Halorientalis*, *Halonotius*, *Natronomonas*, and *Haloglomus*, forming strongly supported clades with reference sequences retrieved from GenBank. Sample-specific OTUs (e.g., S1, S2, S3, S5, and S6) grouped consistently with their closest taxonomic relatives, confirming their phylogenetic placement. In addition, several bacterial taxa, such as *Enhydrobacter*, *Faucicola*, *Salinibacter*, and *Aliifodinibius*, formed distinct bacterial clades clearly separated from archaeal lineages. Bootstrap values indicated strong support for most internal nodes, supporting the robustness of the inferred phylogenetic relationships.

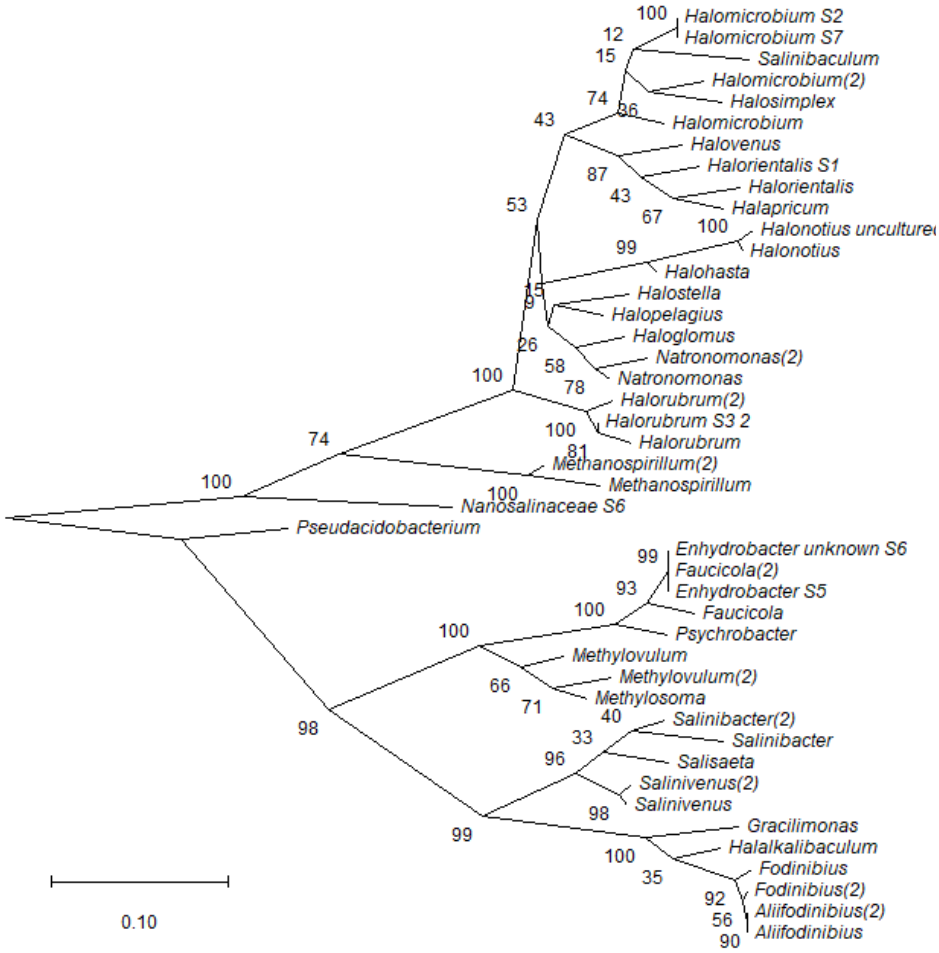

Fig. 7. Maximum Likelihood phylogenetic tree based on partial 16S rRNA gene sequences showing the relationships between dominant OTUs from hypersaline samples and closely related reference taxa retrieved from GenBank. Bootstrap values (>50%, 1,000 replicates) are shown at branch nodes. The scale bar represents the number of nucleotide substitutions per site.

DISCUSSION

Extreme salinity and high ion concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+}) strongly shaped microbial community composition, favoring halophilic archaea. Halobacterota dominated (47%), consistent with their role in sulfur cycling, as suggested by elevated sediment sulfur levels (Mo *et al.*, 2020). Nanohaloarchaeota (21%) and Nanoarchaeota (16%) were also abundant, reflecting adaptation to hypersaline conditions and potential symbiotic interactions with other microbes (Ghuneim *et al.*, 2018). Other archaeal phyla were detected at low abundance, highlighting unexplored microbial diversity within these extreme environments.

Bacterial communities were taxonomically richer but exhibited lower dominance. Major phyla included Bacteroidota, Proteobacteria, Firmicutes, and Desulfobacterota, which contribute to organic matter degradation, carbon cycling, and biogeochemical processes (Hacène *et al.*, 2004; Anantharaman *et al.*, 2018; Menasria *et al.*, 2019; Liu *et al.*, 2023). Families such as Balneolaceae and Moraxellaceae were consistently detected across sites, while genera like Halorientalis, Halomicrobium, Halonotius, and Halorubrum exhibited niche specialization, indicating microhabitat-driven selection.

Alpha diversity and SHE analyses demonstrated that reductions in Shannon diversity at S3 were primarily driven by decreases in evenness rather than species loss ($\ln E = -2.03$), suggesting that environmental stress promotes dominance by specialized taxa while maintaining overall richness. These patterns indicate that dominance effects, rather than richness per se, govern microbial diversity in hypersaline environments, which may influence ecosystem functioning and resilience (Logares *et al.*, 2012). Whittaker's beta diversity value ($\beta w = 2.02$) indicates substantial species turnover among the hypersaline samples, which is consistent with the clear separation patterns observed in the Bray–Curtis–based PCoA ordination, particularly the distinct positioning of sample S3 and the secondary differentiation of samples S5 and S6.

Comparisons with other hypersaline environments suggest that Hadjer El-Melh exhibits both common and unique microbial patterns. The dominance of Halobacterota and the presence of Bacteroidota and Proteobacteria are consistent broadly with those observed in Mediterranean and North African hypersaline lakes (Shokralla *et al.*, 2012; Fernández *et al.*, 2014; Naghoni *et al.*, 2017; Oueriaghli *et al.*, 2018). The relatively high proportion of Nanohaloarchaeota and specialized bacterial genera

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 35, issue 3, 2025, pp. 95 – 105

indicates site-specific adaptations, possibly driven by local ion composition and microhabitat heterogeneity.

The dominance of specialized halophilic taxa, coupled with a stable archaeal core microbiome, underscores the functional importance of these communities in biogeochemical cycling, particularly sulfur and carbon turnover. Moreover, the observed patterns highlight the potential of microbial communities as bioindicators for monitoring hypersaline ecosystem health and environmental change. Rare taxa may also represent a reservoir of biotechnologically valuable extremozymes, further emphasizing the ecological and applied significance of these communities.

Overall, our findings demonstrate that environmental gradients, particularly salinity and ionic composition, strongly influence microbial diversity and community structure. Extreme conditions promote dominance by halophilic specialists, while more moderate sites allow coexistence of diverse archaeal and bacterial taxa, illustrating how both deterministic (environmental filtering) and stochastic processes (microhabitat variability) shape microbial assemblages in hypersaline ecosystems.

CONCLUSION

This study provides the first comprehensive assessment of microbial diversity in Hadjer El-Melh (Djelfa) using 16S rRNA gene sequencing. Both archaeal and bacterial communities were abundant and diverse, with archaea dominated by Halobacterota and Nanohaloarchaeota, and bacteria primarily represented by Bacteroidota and Proteobacteria. Spatial patterns revealed that extreme hypersaline conditions promoted dominance by specialized halophilic taxa, while sites with moderate salinity supported higher microbial richness and evenness. These results are consistent with previous studies on Algerian hypersaline environments while revealing significant variations and providing novel insights into microbial community assembly, dominance patterns, and the environmental drivers shaping salt-rich ecosystems. This study enhances understanding of hypersaline microbial ecology, offering a baseline for future ecological monitoring and conservation. Additionally, the identification of rare and specialized taxa highlights the potential of Hadjer El-Melh as a reservoir of microbes with biotechnological applications, particularly extremozymes and other functional biomolecules.

AUTHOR CONTRIBUTIONS

Conceptualization: S.B.; Methodology: S.B., M.M.B.; Resources: A.B., A.B.H.; Investigation: B.B., F.A.; Data Collection: A.B., A.B.H., B.B.; Data Processing: M.M.B., A.B.; Writing—Original Draft Preparation: A.B.; Data Validation, Writing—Review & Editing: Y.K.K., M.B-B.

FUNDING

This research was funded by the Directorate General for Scientific Research and Technological Development (DGRSDT), Algeria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Directorate General for Scientific Research and Technological Development (DGRSDT), Algeria, for funding this research, and the College of Medicine, Chungbuk National University, South Korea, for hosting and providing laboratory facilities during this study.

REFERENCES

Anantharaman K, Hausmann B, Jungbluth SP, Kantor RS, Lavy A, Warren LA, Rappé MS, Pester M, Loy A, Thomas BC, Banfield JF, Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. ISME Journal, 12(7), 1715–1728, 2018. https://doi.org/10.1038/s41396-018-0078-0

Beddal A, Boutaiba S, Laassami A, Hamaidi F, Enache M, Characterization by polyphasic approach of some indigenous halophilic archaea of Djelfa's rock salt "Hadjr el Meelh", Algeria. Iranian Journal of Microbiology, 14(4), 535–544, 2022. https://doi.org/10.18502/ijm.v14i4.10240

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J, Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome, 6(1), 1–17, 2018. https://doi.org/10.1186/s40168-018-0470-z

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, *et al.*, Interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37, 852–857, 2019. https://doi.org/10.1038/s41587-019-0209-9

Boutaiba S, Hacene H, Bidle KA, Maupin-Furlow JA, Microbial diversity of the hypersaline Sidi Ameur and Himalatt Salt Lakes of the Algerian Sahara. Journal of Arid Environments, 75(10), 909–916, 2011. https://doi.org/10.1016/j.jaridenv.2011.04.010

Bouteldjaoui F, Bessenasse M, Kettab A, Scheytt T, Combining geology, hydrogeology and groundwater flow for the assessment of groundwater in the Zahrez Basin, Algeria. Arabian Journal of Geosciences, 12(24), 1–15, 2019. https://doi.org/10.1007/s12517-019-4948-1

Bunce LE, Lowenstein TK, Jagniecki E, Collins D, Significance of Spring Inflow to Great Salt Lake, Utah, USA. Hydrology, 12(6), 1–19, 2025.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP, DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583, 2016. https://doi.org/10.1038/nmeth.3869

Canganella F, Wiegel J, Extremophiles: from abyssal to terrestrial ecosystems and possibly beyond. Naturwissenschaften, 98(4), 253–279, 2011.

Chao J, Li Z, Sun Y, Aluko OO, Wu X, Wang Q, Liu G, MG2C: A user-friendly online tool for drawing genetic maps. Molecular Horticulture, 1(1), 16, 2021.

Cowan DA, Albers SV, Antranikian G, Atomi H, Averhoff B, Basen M, Driessen AJM, Jebbar M, Kelman Z, Kerou M, Littlechild J, Müller V, Schönheit P, Siebers B, Vorgias K, Extremophiles in a changing world. Extremophiles, 28(2), 1–24, 2024. https://doi.org/10.1007/s00792-024-01341-7

Edbeib MF, Wahab RA, Huyop F, Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments. World Journal of Microbiology and Biotechnology, 32(8), 1–23, 2016. https://doi.org/10.1007/s11274-016-2081-9

Fernández AB, Vera-Gargallo B, Sánchez-Porro C, Ghai R, Papke RT, Rodriguez-Valera F, Ventosa A, Comparison of prokaryotic community structure from Mediterranean and Atlantic saltern concentrator ponds by a metagenomic approach. Frontiers in Microbiology, 5(196), 1–12, 2014. https://doi.org/10.3389/fmicb.2014.00196

Gavrieli I, Beyth M, Yechieli Y, The dead sea: A terminal. *In*: microbiology and biogeochemistry of hypersaline environments, 5, 121–133, 1998.

Ghuneim LAJ, Jones DL, Golyshin PN, Golyshina OV, Nano-sized and filterable bacteria and archaea: Biodiversity and function. Frontiers in Microbiology, 9(1971), 1–12, 2018. https://doi.org/10.3389/fmicb.2018.01971

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ, Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rDNA-based microbial community composition. Applied and Environmental Microbiology, 66(12), 5488–5491, 2000. https://doi.org/10.1128/AEM.66.12.5488-5491.2000

Hacène H, Rafa F, Chebhouni N, Boutaiba S, Bhatnagar T, Baratti JC, Ollivier B, Biodiversity of prokaryotic microflora in El Golea Salt lake, Algerian Sahara. Journal of Arid Environments, 58(3), 273–284, 2004. https://doi.org/10.1016/j.jaridenv.2003.08.006

Hayek LC, Buzas MA, Surveying Natural Populations: Quantitative Tools for Assessing Biodiversity. Columbia University Press, New York, 1–563, 1997.

Imhoff JF, Osmoregulation and compatible solutes in eubacteria. FEMS Microbiology Reviews, 2(1-2), 57–66, 1986.

Khlaifat A, Al-Khashman O, Qutob H, Physical and chemical characterization of Dead Sea mud. Materials Characterization, 61(5), 564–568, 2010.

Liu YH, Mohamad OAA, Gao L, Xie YG, Abdugheni R, Huang Y, Li L, Fang BZ, Li WJ, Sediment prokaryotic microbial community and potential biogeochemical cycle from saline lakes shaped by habitat. Microbiological Research, 270, 127342, 2023. https://doi.org/10.1016/j.micres.2023.127342

Logares R, Lindström ES, Langenheder S, Logue JB, Paterson H, Laybourn-Parry J, Rengefors K, Tranvik L, Bertilsson S, Biogeography of bacterial communities exposed to progressive long-term environmental change. The ISME Journal, 7(5), 937–948, 2012. https://doi.org/10.1038/ismej.2012.168

Ma Y, Galinski EA, Grant WD, Oren A, Ventosa A, Halophiles 2010: Life in saline environments. Applied and Environmental Microbiology, 76(21), 6971–6981, 2010. https://doi.org/10.1128/AEM.01868-10

Madsen EL, Microorganisms and their roles in fundamental biogeochemical cycles. Current Opinion in Biotechnology, 22(3), 456–464, 2011.

Mahmoud AM, Ali MH, Abdelkarim MS, Al-Afify AD, Chemical, biochemical, and bioactivity studies on some soda lakes, Wadi El-Natrun, Egypt. Environmental Monitoring and Assessment, 196(5), 436, 2024.

Martínez GM, Pire C, Martínez-Espinosa RM, Hypersaline environments as natural sources of microbes with potential applications in biotechnology: The case of solar evaporation systems to produce salt in Alicante County (Spain). Current Research in Microbial Sciences, 3, 100136, 2022. https://doi.org/10.1016/j.crmicr.2022.100136

Martínez-Alvarez L, Ramond JB, Vikram S, León-Sobrino C, Maggs-Kölling G, Cowan DA, With a pinch of salt: metagenomic insights into Namib Desert salt pan microbial mats and halites reveal functionally adapted and competitive communities. mSystems, 28(12), e00629-23, 2022. https://doi.org/10.1101/2022.02.18.481119

McGonigle JM, Bernau JA, Bowen BB, Brazelton WJ, Metabolic Potential of Microbial Communities in the Hypersaline Sediments of the Bonneville Salt Flats. mSystems, 7(6), e00846-22, 2021. https://doi.org/10.1101/2021.10.18.464844

McMurdie PJ, Holmes S, phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE, 8(4), e61217, 2013.

Menasria T, Aguilera M, Hocine H, Benammar L, Ayachi A, Si Bachir A, Dekak A, Monteoliva-Sánchez M, Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes. Microbiological Research, 207, 289–298, 2018. https://doi.org/10.1016/j.micres.2017.12.011

Mo S, Li J, Li B, Nie S, Zhang Z, Kashif M, He S, Liao J, Jiang Q, Shen P, Yan B, Jiang C, Impacts of Crenarchaeota and Halobacterota on sulfate reduction in the subtropical mangrove ecosystem as revealed by SMDB analysis. bioRxiv, 2020.08.16.252635, 2020.

https://doi.org/10.1101/2020.08.16.252635

Naghoni A, Emtiazi G, Amoozegar MA, Cretoiu MS, Stal LJ, Etemadi M, Peymani A, Microbial diversity in the hypersaline Lake Meyghan, Iran. Scientific Reports, 7, 11522, 2017. https://doi.org/10.1038/s41598-017-11585-3

Oren A, Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. Journal of Industrial Microbiology and Biotechnology, 28(1), 56–63, 2002.

Oueriaghli N, Castro DJ, Llamas I, Béjar V, Martínez-Checa F, Study of bacterial community composition and correlation of environmental variables in Rambla Salada, a hypersaline environment in South-Eastern Spain. Frontiers in Microbiology, 9, 1377, 2018.

Pikuta EV, Hoover RB, Tang J, Microbial extremophiles at the limits of life. Critical Reviews in Microbiology, 33(3), 183–209, 2007.

Poli A, Di Donato P, Abbamondi GR, Nicolaus B, Synthesis, production, and biotechnological applications of exopolysaccharides and polyhydroxyalkanoates by archaea. Archaea, 2011, 693253, 2011.

Post FJ, Microbiology of the Great Salt Lake north arm. *In*: Salt lakes: Proceedings of the international symposium on Athalassic (Inland) salt lakes, held at Adelaide, Australia, October 1979, 59–69, 1981.

Rabehi SA, Rezzaz MA, Kherrour L, Souiher K, Towards the Development of Ecotourism in the Ouled-Nail Mountains: a Case Study of the Djelfa Municipality in Algeria. Geojournal of Tourism and Geosites, 53(4), 1411–1429, 2023. https://doi.org/10.30892/gtg.50422-1140

Sahli K, Gomri MA, Esclapez J, Gómez-Villegas P, Bonete MJ, León R, Kharroub K, Characterization and biological activities of carotenoids produced by three haloarchaeal strains isolated from Algerian salt lakes. Archives of Microbiology, 204(1), 6, 2022.

Shannon CE, A mathematical theory of communication. The Bell System Technical Journal, 27(3), 379–423, 1948.

Sheikheh S, Rabiei M, Rasouli V, A Review of Evaporite Beds Potential for Storage Caverns: Uncovering New Opportunities. Applied Sciences, 15(9), 4685, 2025. https://doi.org/10.3390/app15094685

Shokralla S, Spall JL, Gibson JF, Hajibabaei M, Next-generation sequencing technologies for environmental DNA research. Molecular Ecology, 21(8), 1794–1805, 2012. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Simpson EH, Measurement of diversity. Nature, 163(4148), 688, 1949.

Taher AG, Microbially induced sedimentary structures in evaporite–siliciclastic sediments of Ras Gemsa sabkha, Red Sea Coast, Egypt. Journal of Advanced Research, 5(5), 577–586, 2014. https://doi.org/10.1016/j.jare.2013.07.009

Van den Burg B, Extremophiles as a source for novel enzymes. Current Opinion in Microbiology, 6(3), 213–218, 2003. https://doi.org/10.1016/S1369-5274(03)00060-2

Wang Y, Li W, Bao G, Bai M, Ye H, Differences in archaeal diversity and potential ecological functions between saline and hypersaline lakes on Qinghai-Tibet Plateau were driven by multiple environmental and non-environmental factors beyond the salinity. BMC Microbiology, 24(1), 153, 2024.

Warren JK, Interpreting Evaporite Textures. In: Evaporites: A Geological Compendium, 1–83, 2016. https://doi.org/10.1007/978-3-319-13512-0_1

Youssef NH, Ashlock-Savage KN, Elshahed MS, Phylogenetic diversities and community structure of members of the extremely halophilic Archaea (order Halobacteriales) in multiple saline sediment habitats. Applied and Environmental Microbiology, 78(5), 1332–1344, 2012. https://doi.org/10.1128/AEM.07420-11

Zhang J, Zhang C, Quality assurance/quality control in surface water sampling. *In*: quality assurance & quality control of environmental field sampling, Future Science Ltd, 76–96, 2014. https://doi.org/10.4155/ebo.13.371