Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XV, 2026
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

SALT LAKE MICROBIAL ECOSYSTEMS: IMPORTANCE AND CONSERVATION CHALLENGES

Lucia-Roxana COJOC, Mirela-Mădălina MOLDOVEANU, Larisa-Isabela FLORESCU, Mădălin-Iancu ENACHE, Anca-Ioana LUCACI

Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, District 6, 060031, Bucharest, Romania

Corresponding author email: mirela.moldoveanu@ibiol.ro

Abstract

High salinity lakes harbor halophilic microbial communities with specialized adaptations to osmotic stress. These ecosystems provide important ecological services and biotechnological potential, yet they face increasing threats from climate change and human activities. Understanding their microbial diversity is essential for conservation efforts. The present study aims to characterize cultivable halophiles from three saline lakes located in Durgau-Valea Sarata area, documenting their diversity, salt tolerance and capacity to produce extracellular enzymes.
XRF analysis revealed silica, chloride, calcium and titanium throughout all samples, with sporadic traces of bromide, potassium and sulfur. Cultivation on media with different salt concentrations yielded a total of 60 isolates across the three lakes, optimizing recovery of diverse halophiles including both archaea and bacteria. 16S rRNA gene sequencing of representative isolates revealed affiliation to Salinivibrio, Halomonas, Chromohalobacter, Marinococcus, Salicola, Halococcus, Halobacterium, Haloarcula and Halorubrum genera. Bacterial isolates produced exoenzymes (esterase, inulinase, amylase) and five strains demonstrated biofilm formation capacity.
Our results provide reference data on diversity of cultivable halophiles and functional traits in these threatened saline ecosystems.

Key words: *halophiles, anthropo-saline lakes, biofilm formation, cultivable diversity, exoenzymes.*

INTRODUCTION

Hypersaline habitats shelter diverse microbial communities, including extreme halophiles, moderate halophiles, and halotolerant microorganisms, which have developed strategies to overcome osmotic stress and elevated salinity levels through mechanisms such as accumulation of compatible solutes and efficient ion transport systems (Quesada et al., 1983). Recent studies have revealed that these communities are characterized by specific trophic interactions shaped by high levels of specialization (Saccò et al., 2021).
Hypersaline lakes, which account for almost half (44%) of inland water volume (Saccò et al., 2021), exhibit uniqueness not only in their extreme physicochemical conditions but also in the diversity and metabolic potential of their microbial communities (Paul & Mormile, 2017). These ecosystems offer biotechnological potential, therapeutic uses through heliothermic properties and organic-rich sediments (Alexe et al., 2018; Rus et al., 2020), and microbiological research opportunities, where microbial communities serve as environmental indicators (Oren, 2018; Paul & Mormile, 2017).
However, hypersaline lakes are highly susceptible to climate change, as increased temperatures lead to high evaporation rates, further intensifying salinity levels that challenge microbial survival (Paul & Mormile, 2017), while rising lake surface temperatures affect ecosystem structure and metabolic activities (Dokulil et al., 2021). Since salinity drives microbial diversity, alterations to this foundation affect the entire food web (Baxter & Butler, 2020). Additionally, anthropogenic pressures, particularly intense touristic exploitation through uncontrolled bathing, cause degradation and diminishment of the heliotherm phenomenon (Alexe & Furtună, 2012). Continued loss and degradation of hypersaline habitats will result in the loss of taxonomic and functional diversity, with impacts at multiple scales, making the prevention of ecosystem destruction and the development of sustainable use strategies a pressing issue (Saccò et al., 2021).

Salt lakes in Transylvania are distinctive components of the basin landscape formed by salt dissolution of underground halite deposits (Har et al., 2010; Alexe et al., 2018). These lakes are small and quite deep, offering ideal conditions for stratification and the development of heliothermy (Mera et al., 2010; Sonnenfeld & Hudec, 1980). Previous microbiological research in the Turda region has focused mainly on the characterization of halophilic bacterial communities associated with Turda Salt Mine (Carpa et al., 2014). Several studies have also investigated the microbial diversity of hypersaline meromictic lakes from the Transylvanian Basin, with particular emphasis on archaeal communities and their vertical distribution in the water column (Andrei et al., 2015; Baricz et al., 2014; 2015). Nevertheless, information regarding the diversity of cultivable bacterial populations inhabiting these saline lakes remains relatively limited.

Understanding the composition and physiological capabilities of halophilic microbial communities is essential for both conservation efforts and potential biotechnological exploitation. The present study aims to document the diversity and physiological characteristics of cultivable halophilic microorganisms from three saline lakes in the Durgău-Valea Sărată area (Turda, Romania). By characterizing these communities, their salt tolerance profiles, and enzymatic capabilities, this research provides baseline data necessary for conservation and monitoring as environmental pressures from climate change and anthropogenic activities intensify.

MATERIALS AND METHODS

Site description

Water samples were collected from three saline lakes in the Durgău-Valea Sărată area of Turda (46°35'16.8"N, 23°47'14.1"E), Cluj County, Romania (Figure 1). This area is part of Sărăturile Ocna Veche, a protected area of national interest corresponding to IUCN category IV (mixed nature reserve). The salt lakes in Turda developed over the sites of old salt mine chambers used during the 15th and 16th centuries (Mera et al., 2010). These lakes are characterized by stratification (formation of permanent density water layers) and heliothermy, with a surface freshwater layer of variable thickness (0.15 to 0.2 m) (Mera et al., 2010).

Ocnei Lake emerged around 1800 following the collapse of the "Ocna cea Mare" excavation (Mera et al., 2010). This anthropo-saline lake contains chlorinated-sodic mineral waters with mineralization ranging between 19.27 and 225 mg L^{-1} (Rus et al., 2020) and surface salinity varying from 1.78 to 40.42 g Cl/l depending on freshwater input (Munteanu & Munteanu, 2012). The lake is actively used for recreation and therapy, with heavy exploitation during summer since 2009 (Avram et al., 2022). Over time, maximum temperatures in the heliothermic layer have decreased, mainly due to intensive exploitation of the waters for bathing and treatment activities (Alexe & Furtună, 2012).

Rotund Lake was created by the collapse of the "Ocna cea Mica" excavation (Mera et al., 2010). Similar to Ocnei Lake, it is an anthropo-saline lake used for recreation and therapy (Avram et al., 2022), with chlorinated-sodic mineral waters (Rus et al., 2020).

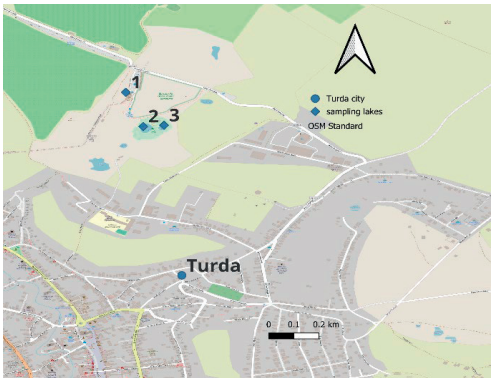


Figure 1. The map with sampling points (QGIS Desktop 3.40.15 with OSM standard)

Therezia Lake is a subterranean lake located at 112 m depth in the Turda Salt Mine, with a water depth of 0.5- 8 m and occupying approximately 80% of the exploitation chamber surface (Kimic et al., 2021). The lake formed through human-induced geomorphological processes: the Theresia chamber, originally a salt extraction site named in honor of Empress Maria Theresa, became structurally unstable following

extensive salt extraction and ultimately collapsed, allowing surface water infiltration that gradually flooded the cavity (Avram et al., 2022). A central island formed by residual salt accumulated after the cessation of extraction activities in 1880 (Kimic et al., 2021). The lake is characterized by a slightly alkaline pH (8.7), specific conductance of 114 mS cm^{-1}, and estimated salinity of 90.98 g L^{-1} (Carpa et al., 2014). The subterranean environment maintains relatively constant temperatures (11.8-12.2°C) and humidity (67-70%) with no air flow (Enache, 2010).

Sampling

The water samples were collected from the surface layers of the three lakes, about 0.5 m beneath the water surface. The sampling procedure was performed aseptically with sterile utensils and sterilized collection containers, thereby avoiding external contamination. Samples were kept refrigerated and were transported in cooler storage boxes to the laboratory for further chemical and microbiological analysis.

XRF analysis

The chemical composition of the water samples was determined using a Supermini X-Ray Fluorescence Spectrometer (Rigaku Corporation, Japan), following the manufacturer's semiquantitative method for light element analysis in a helium atmosphere. SQX software was used to calculate elemental oxide mass percentages (mass %). Weighed 10 mL volumes of each sample were used for analysis.

Isolation of halophilic microorganisms and estimation of total CFU number was carried on 3 different nutritive media, with the following compositions (g L^{-1}): MH medium - yeast extract, 10; proteose peptone, 5; glucose, 1; NaCl, 100; $MgCl_2$ x $6H_2O$, 7; $MgSO_4$ x $7H_2O$, 9.6; $CaCl_2$ x $2H_2O$, 0.36; KCl, 2; $NaHCO_3$, 0.06; NaBr, 0.026 (Quesada et al., 1983), JCM 168 medium (Japan Collection of Microorganisms) - casamino acids, 5; yeast extract, 5; sodium glutamate, 1; trisodium citrate, 3; $MgSO_4 \cdot 7H_2O$, 20; KCl, 2; NaCl, 200; $FeCl_2 \cdot 4H_2O$, 36; $MnCl_2 \cdot 4H_2O$, 0.36, MR medium - NaCl, 250; $MgCl_2$ $6H_2O$, 13; $MgSO_4$ $7H_2O$, 20; KCl, 4; $CaCl_2$ $2H_2O$, 1; NaBr, 0.5; $NaHCO_3$, 0.2; yeast extract, 5; tryptone, 8; and glucose, 1 (Hedi et al., 2009). A quantity of 20.0 g L^{-1} agar was added to each type of medium for solidification, and the pH was adjusted to 7.2 before autoclaving.

In order to estimate the total CFU number in each sample and isolate the halophilic microorganisms in pure cultures, the serial dilutions method was applied, followed by the pour-plate technique and 3 streak-plate purification passages. The experiments were conducted in triplicate. The cultures incubation was performed at 30°C (MH) or 37°C (JCM 168 and MR) for a period of 30 days.

The susceptibility to inhibitors (antibiotics/ bile salts) of the new isolates was tested in order to confirm assignment to the *Bacteria* domain and to differentiate them from halophilic archaea. Two media variants with specific inhibitors were used for this purpose: one containing 0.002% chloramphenicol and the other containing 0.004% sodium deoxycholate. Strains that grew in the presence of the bile salt were considered halophilic bacteria.

Establishing the salinity range

The range of salt concentration that allows bacterial growth was determined in MH, JCM168, and MR media containing different NaCl concentrations: 0, 1, 2, 3, 4, and 5 M. The halophilic isolates were classified based on the most widely accepted system defined by Kushner & Kamekura (1988).

Assessment of extracellular enzyme production

MH medium with NaCl concentrations adjusted according to previously obtained results was used for establishing the ability of selected isolates to produce extracellular hydrolytic enzymes (amylase, esterase, inulinase). This was qualitatively assessed on solid basal MH (without proteose peptone and glucose) to which the interest substrate was added.

For the amylolytic activity, 2 g L^{-1} starch was added to the basal medium. The selected isolates were inoculated on the surface of agar plates, incubated at 30°C for 48 hours and subsequently flooded with I_2 – KI solution (0.1% I_2 – 0.2% KI). The presence of a clear zone around the

culture spot indicates the starch hydrolysis and strains were recorded positive for amylase production (Ali et al., 2024).

To determine the esterase activity, the basal medium was supplemented with 0.01% $CaCl_2$. After autoclaving, sterile Tween 80 was added to a final concentration of 1% (v/v). Positive reaction was indicated by the appearance of a white precipitate formed around the growth areas of the bacterial cultures (Ali et al., 2024).

For cellulase activity, basal medium was supplemented with 0.5 g L^{-1} carboxymethylcellulose (CMC). After incubating the inoculated plates at 30°C for 48 hours, they were flooded with 0.1% Congo Red solution for 15 minutes. The dyeing agent was removed by rinsing with 1M NaCl for 5-10 minutes (Rohban et al., 2009; Bambharolia et al., 2024).

The production of inulinase by the investigated isolates was detected by adding 2 g L^{-1} inulin to basal medium. Bacterial growth after 48 hours of incubation at 30°C was considered a positive result (Rohban et al., 2009).

Molecular identification of the isolates

Total genomic DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen), according to the manufacturer's protocol. 16S rRNA gene was PCR amplified with the pair of primers 27F (AGAGTTTGATCCTGGCTCAG) – 1492R (GGTTACCTTGTTACGACTT) for bacteria (Miller et al., 2013), while for archaea, the set of primers 20F (AGTTTGATCATGGCTCA) – 1540R (AAGGAGGTGATCCAACCGCA) (Ohnishi et al., 2011) was used. Amplicons were analyzed by agarose gel electrophoresis (1% w/v) with Red Safe staining and visualized under UV light against O'GeneRuler 1Kb Plus DNA Ladder (Thermo Scientific). Following DNA sequencing performed by CeMIA SA (Larissa, Greece), the resulting nucleotide sequences were compared using the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov/BLAST/).

Biofilm production was assessed in five bacterial isolates selected for mucoid/slimy colony appearance, which typically indicates extracellular polysaccharide production.

The experiment was carried out in 96-well U-bottom microplates with following the method described by O'Toole (2011). The experiment was performed with 5 replicate wells per isolate. Plates were incubated for 31 days in static conditions, at 30ºC, and the biofilm formed was quantified periodically by reading the absorbance at 550 nm (BMG LABTECH FLUOstar Omega microplate reader).

RESULTS AND DISCUSSIONS

XRF analysis

Generally, silica, chloride, sodium, calcium and titanium were present in all investigated samples, with slight quantitative variation according to water source. Sporadic traces of bromide, potassium and sulfur were also detected according to data presented in Figure 2. The considerably smaller amounts of silica detected in the Therezia Lake, could be associated with the position of the lake inside the salt mine, unlike the other two surface lakes positioned in open space. As previously stated, silica should not be considered native ions in the salt massifs (Cojoc et al., 2013).

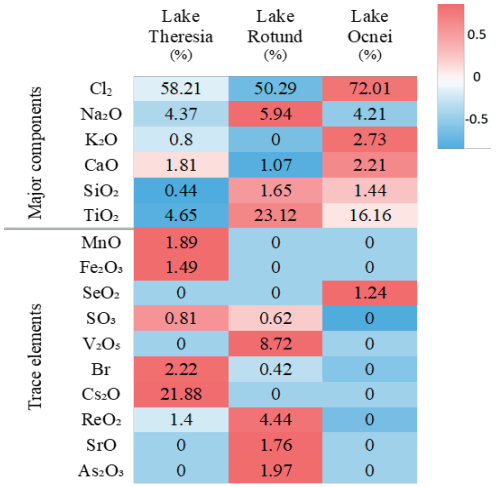


Figure 2. Heatmap of XRF-determined chemical composition of the three salt lakes. Color intensity corresponds to row-wise Z-score normalized values representing relative abundance from low (light) to high (dark). Values indicate measured oxide concentrations (mass %)

High concentrations of titanium and vanadium, both toxic to aquatic organisms and humans, suggest anthropogenic contamination from industrial activities, pigments, cosmetics, and

wastewater entering the lakes via surface runoff or wind (Catana et al., 2022).
The high chlorine concentration observed in all three investigated lakes is reflected in the water's salinity. Sodium chloride, which is a thermophile molecule contributes to an increase in salinity of the warm layer during the daytime (Sonnenfeld & Hudec, 1980).
The performed microbiological analyses proved that the investigated lakes are highly populated by halophilic microorganisms belonging to the *Bacteria* and *Archaea* domains.

Estimation of the total CFU number and the isolation of halophilic microorganisms
After 48h of incubation, the number of CFU mL^{-1} on MH medium was estimated as follows: Ocnei Lake – 4.7 x 10^2 CFU mL^{-1}, Therezia Lake – 0 CFU mL^{-1}, Rotund Lake – 1.26 x 10^2 CFU mL^{-1}. No colonies grew on JCM 168 and MR media after 48 hours of incubation at 37ºC.
Pink-red and white-cream colonies developed on JCM 168 medium only after 30 days of incubation. The number of CFU mL^{-1} was estimated as follows: Ocnei Lake – 10 CFU mL^{-1}, Therezia Lake – 82.33 CFU mL^{-1}, Rotund Lake – 13 CFU mL^{-1}. On MR medium pink, yellow, and orange colonies developed after 1 month of incubation only from the Therezia Lake sample, the estimated CFU number for this sample being 20 CFU mL^{-1}.
A total number of 60 isolates across the three investigated lakes (13 from Ocnei Lake, 25 from Therezia Lake and 22 from Rotund Lake) were obtained and further purified.

The inhibitor tests performed showed that all the 25 microbial isolates obtained on MH medium belonged to the *Bacteria* domain, being able to grow in the presence of deoxycholic acid sodium salt.
On JCM 168 medium, 14 of the obtained isolates showed susceptibility to bile salts and were also positive for lysis in sterile distilled water after incubation for one hour at room temperature. The susceptible isolates were thus classified as archaea. The remaining 15 strains resistant to bile salts were selected for further experiments and considered to belong to the *Bacteria* domain. Following the same differentiation test, two of the isolates obtained on MR medium were classified as bacteria and 4 were assigned to *Archaea.* Across the three investigated lakes different domain distributions of the isolates were observed (Figure 3).

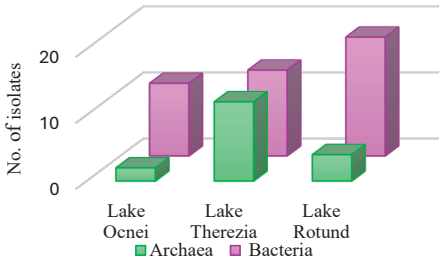


Figure 3. Domain distribution of isolates obtained from the saline lakes

Establishing the salinity range
Based on optimal salinity for growth, this study revealed distinct halophilic profiles between surface and subterranean environments.
The two surface anthropo-saline lakes - Ocnei and Rotund - were dominated by halotolerant strains (46% and 61% of the isolates), with moderately halophilic isolates representing 36% and 39%, respectively. Extremely halophilic strains were detected only in Ocnei Lake (18%), whereas they were absent from Rotund Lake. In contrast, the subterranean Therezia Lake exhibited a distinct distribution, with predominant moderately halophilic strains (46%), followed by halotolerant (39%) and extremely halophilic (15%) representatives, suggesting different selective pressures in the isolated subterranean environment.

Assessing of the capacity of producing extracellular enzymes
Screening of the bacterial isolates revealed a heterogeneous distribution of extracellular enzymatic capabilities. A considerable number of strains expressed lipolytic (5 isolates) and proteolytic activity (6 isolates), whereas inulin degradation was detected only in three isolates. CMC was not hydrolyzed by any of the isolates. The capacity to degrade complex carbohydrates was less prevalent. Amylolytic activity was detected in a limited number of isolates and showed only partial overlap with lipolytic activity.
A smaller number of isolates (5) demonstrated combined hydrolytic activities, suggesting a

high versatility. The presence of such polyvalent strains may reflect ecological adaptation to fluctuating nutrient availability, highlighting potential candidates for saline bioprocesses.

Molecular identification of the isolates

Agarose gel electrophoresis confirmed successful PCR amplification with domain-specific primers, by yielding products of expected size for both bacterial and archaeal 16S rRNA genes (Figure 4).

Representative isolates from the three investigated anthropo-saline lakes were identified by partial 16S rRNA gene sequencing as belonging to bacterial and archaeal genera as presented in Table 1.

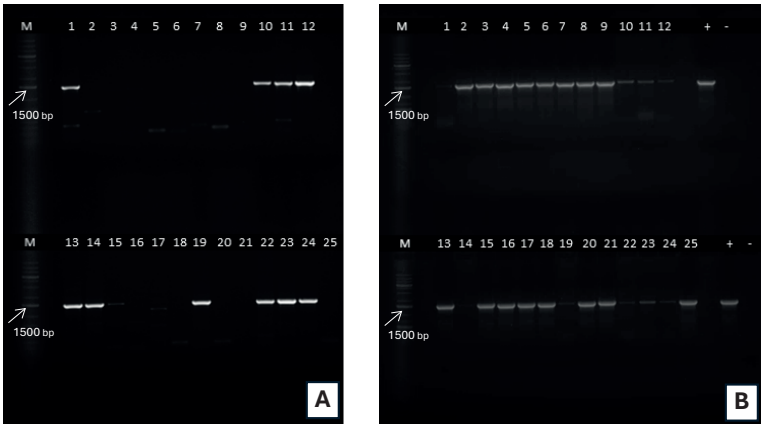


Figure 4. Domain-specific amplification of 16S rRNA genes with (A) Archaeal primers (20F/1540R), (B) Bacterial primers (27F/1492R). Lane identifications: M = 1 kb Plus DNA ladder; 1-25 = isolates from the 3 saline lakes; + = positive control, - = negative control

Table 1. Taxonomic affiliation of sequenced isolates from the three saline lakes

Sample source	Affiliation to bacterial genera	Affiliation to archaeal genera
Ocnei Lake	*Halomonas* (3), *Marinococcus* (1), *Salinivibrio* (1), *Salicola* (1)	*Halococcus* (1)
Therezia Lake	*Halomonas* (1), *Salicola* (1), *Chromohalobacter* (3)	*Haloarcula* (2), *Halococcus* (2), *Halobacterium* (3), *Halorubrum* (1)
Rotund Lake	*Marinococcus* (3)	Not sequenced

*Numbers in parentheses indicate isolates assigned to each genus.

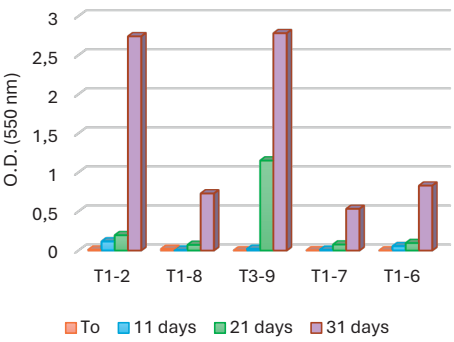


Figure 5. Biofilm production capacity of the investigated bacterial isolates

Biofilm formation capacity

Biofilm production was assessed in five bacterial isolates showing mucoid/slimy colony morphology. All five isolates demonstrated biofilm formation capacity, though with varying kinetics and production levels (Figure 5).

Isolate T3-9 showed a significant capacity for biofilm formation after 21 days of incubation, while T1-2 was able to produce notable biofilms only after 31 days. The remaining three isolates also formed biofilms after 31 days of incubation, but at lower levels than T3-9 and T1-2.

CONCLUSIONS

The three anthropo-saline lakes in the Durgău-Valea Sărată area (Turda, Romania) harbor diverse cultivable halophilic microbial communities comprising both bacterial and archaeal representatives.

Distinct halophilic profiles were observed between surface saline lakes (Ocnei and Rotund) and the subterranean Therezia Lake, with surface lakes dominated by halotolerant strains, while the subterranean environment showed higher proportions of moderately and extremely halophilic microorganisms, suggesting that environmental isolation may create different selective pressures in these habitats.

The presence of elevated titanium and vanadium concentrations detected through XRF analysis indicates anthropogenic contamination associated with intensive bathing activities, particularly from sunscreens and cosmetic product pigments, raising concerns about impacts on microbial community health and ecosystem stability.

The enzymatic capabilities of bacterial isolates, including esterase, inulinase, and amylase production, along with biofilm-forming capacity across multiple strains, highlight the biotechnological potential of halophilic microorganisms from these threatened ecosystems and underscore the economic value of preserving microbial diversity.

This study provides essential baseline data on cultivable halophilic diversity and functional traits in Turda saline lakes, establishing reference information necessary for future monitoring and conservation efforts as these ecosystems face mounting pressures from climate change and anthropogenic activities.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Institute of Biology Bucharest of Romanian Academy and was financed from the projects no. RO1567-IBB05/2026 and RO1567-IBB02/2026.

REFERENCES

Alexe, M., & Furtună, P.-R. (2012). Natural and Anthropic Risks in the Area of Durgău- Valea Sărată Turda Salt Lakes. *Proceedings of the Water Resources and Wetlands, Tulcea, Romania*, 244–247.

Alexe, M., Șerban, G., Baricz, A., Andrei, A.Ș, Cristea, A., P. Battes, K., Cîmpean, M., Momeu, L., Muntean, V., Porav, S. A., & Banciu, H. L. (2018). Limnology and plankton diversity of salt lakes from Transylvanian Basin (Romania): A review. *Journal of Limnology*, 77(1), 17–34. https://doi.org/10.4081/jlimnol.2017.1657.

Ali, A. M., Abdel-Rahman, T. M. A., & Farahat, M. G. (2024). Bioprospecting of Culturable Halophilic Bacteria Isolated from Mediterranean Solar Saltern for Extracellular Halotolerant Enzymes. *Microbiology and Biotechnology Letters, 52*(1), 76–87. https://doi.org/10.48022/mbl.2401.01010.

Andrei, A.-Ș., Robeson, M. S., II, Baricz, A., Coman, C., Muntean, V., Ionescu, A., Etiope, G., Alexe, M., Sicora, C. I., Podar, M., & Banciu, H. L. (2015). Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. *The ISME Journal*, *9*(12), 2642–2656. https://doi.org/10.1038/ismej.2015.60.

Avram, S. E., Rus, L., Micle, V., & Hola, S. S. (2022). Evaluation and Evolution of the Physico-Chemical Parameters of Ocnei and Rotund Lakes Located near the "Salina Turda" Mine, Romania. *Water*, *14*(15). https://doi.org/10.3390/w14152366.

Bambharolia, R. P., Vyas, T. K., Vavdiya, P. A., Damasia, D. M., Garaniya, N. H., & Deshmukh, A. J. (2024). A Comparative Study of Different Staining Techniques for Cellulase Activity on CMC (Carboxy Methyl Cellulose) Agar. *International Journal of Economic Plants*, *11*, 303–309. https://doi.org/10.23910/2/2024.5451b.

Baricz, A., Coman, C., Andrei, A. Ș., Muntean, V., Keresztes, Z. G., Păușan, M., Alexe, M., & Banciu, H. L. (2014). Spatial and temporal distribution of archaeal diversity in meromictic, hypersaline Ocnei Lake (Transylvanian Basin, Romania). *Extremophiles*, *18*(2), 399–413. https://doi.org/10.1007/s00792-013-0625-6.

Baricz, A., Cristea, A., Muntean, V., Teodosiu, G., Andrei, A.-Ș., Molnár, I., Alexe, M., Rakosy-Tican, E., & Banciu, H. L. (2015). Culturable diversity of aerobic halophilic *Archaea* (Fam. Halobacteriaceae) from hypersaline, meromictic Transylvanian lakes. *Extremophiles*, *19*(2), 525–537. https://doi.org/10.1007/s00792-015-0738-1.

Baxter, B. K., & Butler, J. K. (2020). Climate Change and Great Salt Lake. In B. K. Baxter & J. K. Butler (Eds.), *Great Salt Lake Biology: A Terminal Lake in a Time of Change* (pp. 23–52). Springer International Publishing. https://doi.org/10.1007/978-3-030-40352-2_2.

Carpa, R., Keul, A., Muntean, V., & Dobrotă, C. (2014). Characterization of Halophilic Bacterial Communities in Turda Salt Mine (Romania). *Origins of Life and Evolution of the Biosphere*, *44*(3), 223–230. https://doi.org/10.1007/s11084-014-9375-4.

Catana, R. D., Podosu, A., Florescu, L. I., Mihai, R. A., Enache, M., Cojoc, R., & Moldoveanu, M. (2022). Quantitative Analyses of Chemical Elements in *Phragmites australis* as Bioindication of Anthropization in Urban Lakes. *Sustainability*, *15*(1), 553. https://doi.org/10.3390/su15010553.

Cojoc, R., Enache, M., & Codreanu-Balcescu, D. (2013). Approaching the halophilic bacteria presence as possible indicator of biological dynamics in hypersaline aquatic environments. *Drobeta- Seria Stiintele Naturii, XXIII*, 31–39.

Dokulil, M., Eyto, E., Maberly, S., May, L., Weyhenmeyer, G., & Woolway, R. (2021). Increasing maximum lake surface temperature under climate change. *Climatic Change*, *165,* 56. https://doi.org/10.1007/s10584-021-03085-1.

Enache, L. (2010). *The Microclimate, Bioclimate and Natural Air Ionization in Turda Salt Mine – Environmental Factors with Therapeutic Potential*. Masa Rotunda Dedicata Dezvoltării Turismului Balneoclimateric şi Speleoterapiei în Salina Turda.

Har, N., Rusz, O., Vlad, C., & Barbu, O. (2010). New data on the mineralogy of the salt deposit from Sovata (Mureş county-Romania). *Carpathian Journal of Earth and Environmental Sciences*, *5*, 127–135.

Hedi, A., Sadfi, N., Fardeau, M.-L., Rebib, H., Cayol, J.-L., Ollivier, B., & Boudabous, A. (2009). Studies on the Biodiversity of Halophilic Microorganisms Isolated from El-Djerid Salt Lake (Tunisia) under Aerobic Conditions. *International Journal of Microbiology*, 731786. https://doi.org/10.1155/2009/731786.

Kimic, K., Costa, C. S., & Negulescu, M. (2021). Creating Tourism Destinations of Underground Built Heritage—The Cases of Salt Mines in Poland, Portugal, and Romania. *Sustainability*, *13*(17). https://doi.org/10.3390/su13179676.

Kushner, D. J., & Kamekura, M. (1988). Physiology of halophilic Eubacteria. In F. Rodriguez-Valera (Ed.), *Halophilic Bacteria* (Vol. 1, pp. 109–138). CRC Press, Inc., Boca Raton. https://scispace.com/papers/physiology-of-halophilic-eubacteria-3renqnh8jo.

Mera, O., Stefanie, T., & Visinescu, V. (2010). *Cetatea din Muntele de Sare*. Targul Cartii.

Miller, C. S., Handley, K. M., Wrighton, K. C., Frischkorn, K. R., Thomas, B. C., & Banfield, J. F. (2013). Short-Read Assembly of Full-Length 16S Amplicons Reveals Bacterial Diversity in Subsurface Sediments. *PLOS ONE*, *8*(2), e56018. https://doi.org/10.1371/journal.pone.0056018.

Munteanu, C., & Munteanu, D. (2012). *Ape terapeutice clorurate – sodice*. Ed. Balneara.

Ohnishi, A., Abe, S., Nashirozawa, S., Shimada, S., Fujimoto, N., & Suzuki, M. (2011). Development of a 16S rRNA Gene Primer and PCR-Restriction Fragment Length Polymorphism Method for Rapid Detection of Members of the Genus Megasphaera and Species-Level Identification. *Applied and Environmental Microbiology*, *77*(15), 5533–5535. https://doi.org/10.1128/AEM.00359-11.

Oren, A. (2018). Salt Lakes, Climate Change, and Human Impact: A Microbiologist's Perspective. *2018 Air and Water Components of the Environment Conference Proceedings*, 163–170. https://doi.org/10.24193/AWC2018_20.

O'Toole, G. A. (2011). Microtiter Dish Biofilm Formation Assay. *Journal of Visualized Experiments: JoVE*, (47), 2437. https://doi.org/10.3791/2437.

Paul, V. G., & Mormile, M. R. (2017). A case for the protection of saline and hypersaline environments: A microbiological perspective. *FEMS Microbiology Ecology*, *93*(8), 1–14. https://doi.org/10.1093/femsec/fix091.

Quesada, E., Ventosa, A., Rodriguez-Valera, F., Megias, L., & Ramos-Cormenzana, A. (1983). Numerical Taxonomy of Moderately Halophilic Gram-negative Bacteria from Hypersaline Soils. *Microbiology*, *129*(8), 2649–2657. https://doi.org/10.1099/00221287-129-8-2649.

Rohban, R., Amoozegar, M. A., & Ventosa, A. (2009). Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran. *Journal of Industrial Microbiology & Biotechnology*, *36*(3), 333–340. https://doi.org/10.1007/s10295-008-0500-0.

Rus, L., Avram, S.-E., & Micle, V. (2020). Determination and assessments of physicochemical parameters of the water from anthropo-saline lakes located in the protected area "Salina Turda", Romania. *Studia Universitatis Babeş-Bolyai Chemia*, *65*(2), 257–268. https://doi.org/10.24193/subbchem.2020.2.21.

Saccò, M., White, N. E., Harrod, C., Salazar, G., Aguilar, P., Cubillos, C. F., Meredith, K., Baxter, B. K., Oren, A., Anufriieva, E., Shadrin, N., Marambio-Alfaro, Y., Bravo-Naranjo, V., & Allentoft, M. E. (2021). Salt to conserve: A review on the ecology and preservation of hypersaline ecosystems. *Biological Reviews*, *96*(6), 2828–2850. https://doi.org/10.1111/brv.12780.

Sonnenfeld, P., & Hudec, P. P. (1980). Chapter 8 Heliothermal Lakes. In A. Nissenbaum (ed.), *Developments in Sedimentology*, 28, 93–100. Elsevier. https://doi.org/10.1016/S0070-4571(08)70232-2.