

Peer Review

Review of: "The Limits of Life at Extremely Low Water Activity: Lithium-Concentration Ponds in a Solar Saltern (Salar de Atacama, Chile)"

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Peer Review Report – Recommendations for Improvement

The article by Demergasso et al. explores microbial diversity in hypersaline ponds from an industrial lithium concentration process in the Atacama Desert, aiming to assess life's limits under extreme water scarcity. The study reports a water activity (aw) as low as 0.61, with prokaryotic diversity spanning the salinity gradient. Archaeal DNA was identified in lower salinity ponds, while bacterial DNA persisted throughout. Bacterial cDNA was retrieved at aw 0.2, and enrichment cultures confirmed bacterial viability. These findings suggest microbial adaptation to extreme conditions, offering insights into extremophile survival and their potential biotechnological applications.

The study addresses an interesting topic, yet certain aspects could be refined to strengthen its impact.

1. Introduction and Research Focus

The introduction could be more structured to clearly define the study's objectives and its alignment with previous research. Currently, it provides general information on adverse conditions for life—including human survival, which is not revisited later—without establishing specific examples of bacterial diversity in these environments. Clarifying the focus on microbial communities in extreme environments and integrating concrete case studies would enhance the manuscript's coherence.

A more precise discussion of the statement “there are contradictory indications about the presence of life in salt-rich environments” would be beneficial. The manuscript references five studies but does not elaborate on their content or the specific contradictions. A clearer synthesis of these references would help contextualize the research.

2. Relevance and Scope

The study appears to explore microbial communities within an industrial process, yet its broader significance remains somewhat unclear. A stronger emphasis on whether the research primarily seeks to understand microbial adaptations to extreme environments or to examine shifts in community structure due to industrial lithium extraction would help define its impact. If the study aims to contribute to biotechnology, highlighting potential applications for lithium extraction or biomining would enhance its relevance.

Additionally, as samples were collected from industrial processing plants (Albemarle Corp.), discussing potential anthropogenic influences and contamination risks would improve the study's rigor. A brief section addressing possible sources of external interference and how they were mitigated would strengthen the ecological validity of the findings.

3. Experimental Design and Data Presentation

The methodology is well-documented, and the complexity of sample collection is acknowledged. However, the number of figures could be optimized to improve clarity. The inclusion of 12 figures is excessive and is neither sufficiently justified nor thoroughly addressed in the explanation of the results or the discussion. Many of them present data that could be consolidated into tables or supplementary material.

The characterization of physicochemical parameters and ternary diagrams is informative and should remain in the main text. On the contrary, figures related to copy number values and Shannon index diversity metrics might be better suited as supplementary figures. NMDS diagrams, as well as Figures 10 and 11, could also be included as supplementary materials.

Figure 8 presents an interesting finding but would benefit from clearer explanations in the methods, results, and figure legend to ensure accessibility.

Additionally, while the enrichment strategy is a promising approach, its purpose and expected insights are not clearly stated. Expanding on why enrichment was conducted and how it contributes to the study's conclusions would improve its interpretation.

4. Discussion and Literature Integration

The discussion presents new findings but would benefit from a more structured approach. Some claims, such as “in situ growth in the ponds was prevented by a lack of carbon and energy sources and

vitamins rather than by low aw”, are made without direct measurements of these factors or literature support. Including data or references to support such statements would increase their validity.

Additionally, comparative analyses with other salt flats or extreme low-water environments, particularly in the Atacama Desert, are missing. Incorporating previous studies would contextualize the findings within existing knowledge, helping to assess their novelty and significance.

Certain statements, such as “The first reaction any microbiologist will have after seeing these results will be to assume some kind of contamination”, could be rephrased to maintain scientific objectivity. If contamination is a concern, discussing experimental controls and validation methods would be more effective than general speculative remarks.

5. Formatting and Readability

From a technical standpoint, improving figure formatting would enhance readability.

Fonts and sizes across figures should be standardized.

Image resolution should be improved to ensure clarity, particularly in figures with small symbols or complex data representations.

Final Recommendations

The manuscript explores an intriguing topic, and with some refinements, it could provide valuable insights into microbial survival in extreme saline environments. Strengthening the research focus, clarifying the study’s broader implications, optimizing data presentation, and integrating additional literature would significantly enhance its impact. Addressing these aspects could greatly improve the manuscript’s clarity and relevance.

At this stage, further revisions are recommended before considering the study for publication.

Declarations

Potential competing interests: No potential competing interests to declare.