

## 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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The study titled 'The Limits of Life at Extremely Low Water Activity: Lithium-Concentration Ponds in a Solar Saltern (Salar de Atacama, Chile)' by Demgrasso et al. describes the microbial diversity of Archaea and Bacteria in the low water activity brine samples obtained from the lithium-concentration ponds located in Chile. The present study provides interesting and unexpected results on the bacterial and archaeal diversity patterns in low-water activity conditions and increased salinity conditions. Though the study adds a wealth of information to the field of extremophile microbiology, the current draft needs some major revisions.

1. The manuscript as a whole appears unstructured and unorganized. The introduction appears too fragmented, and it's difficult to track the flow of information. The operation of these solar saltern ponds could be explained in detail (if possible with a figure) as this forms the basis of the present study and would help readers correlate the samples with the salinity and other operating conditions.
2. The quality and readability of the figures could be improved. Further, some of the important figures, like supplementary figure 16, describing the overall genus level distribution, could be moved to the main section.
3. In agreement with the other reviewers, the dilution and dialysis procedures for concentrating and separating the biomass could result in the loss of the inherent extremophilic microorganisms. I believe centrifugation of the brine samples could have been a better option for biomass recovery. Again, the usage of conductivity to correlate the salinity doesn't hold well with the samples with high salinities because the salinity negatively correlates with conductivity at high salt

concentrations. A Baume hydrometer or handheld refractometer would have been an ideal tool for measuring salinity in the samples as well as during the dialysis process.

4. Though a great deal of attention has been given to the contamination issues in the reagents, the taxonomic analysis of the Illumina reads obtained from the reagents is interesting. Since the reads belonging to halophilic bacteria like *Halomonas* were found, could it be due to reagent contamination with aerosols if the lab routinely handles high-salinity samples?
5. For cell abundance estimation studies, acridine orange could be a better option than DAPI.
6. It is not clear why the taxonomic analysis results obtained from the amplicon sequencing experiment were not discussed widely at the genus level in spite of obtaining a good number of reads (up to 0.5 million reads). This could have helped us in comparing the culture-independent results against the enrichment results. I feel most of the discussions were centered on the family-level taxonomic classification. More robust statistical tests (Kruskal-Wallis) could have been applied to establish the significance when comparing the microbial diversity of sampling locations.
7. Culture-dependent studies could have utilized halophilic media instead of LB targeted toward the isolation of halophilic microbes. Further, LB has also been shown to be restrictive on bacterial growth rates. I believe the usage of a true halophilic medium could have resulted in the isolation of an even wider variety of microbes.

## Declarations

**Potential competing interests:** No potential competing interests to declare.