

Peer Review

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

Ignacio Ramos-Tapia<sup>1</sup>

1. Universidad del Desarrollo, Chile

The work of Demergasso et al: " The Limits of Life at Extremely Low Water Activity: Lithium-Concentration Ponds in a Solar Saltern (Salar de Atacama, Chile)" evaluates how far along the salinity gradient life could be found, using a chemical, molecular, and meta-taxonomic approach.

The manuscript is well-written, and the work is an important piece to understand how salinity is related to the microbiological component in an industrial environment with extreme salinity conditions.

However, there are several issues that need to be addressed to improve the final manuscript. I will summarize my general concerns and then present a number of more specific recommendations.

The introduction is clear and addresses the main questions to understand where the work is going. However, in my opinion, to keep the purpose of the work in mind for the reader, I would add a final paragraph with the main objective and/or hypothesis. Because the sentence "Here, we analyze a salinity gradient spanning the entire industrial process and determine the bacteria and archaea present from cDNA as well as rRNA and cultures" is very simple for the enormous work done.

As for materials and methods, these are well-detailed and very well-written.

In the taxonomic and phylogenetic analysis section, I have some questions.

- Was only rarefaction used for normalization? Because if statistical analysis is required to compare taxa between salinity or pond gradients, I would recommend the negative binomial distribution as recommended by McMurdie and Holmes.

- The phylogenetic analysis of the sequences is not presented, such as how they were aligned, packages used, etc.
- Were chloroplast and mitochondria sequences eliminated? Please specify, since this can increase alpha diversity.

The results are well-written and clear.

In microbial diversity, a decrease in bacterial richness was observed when Li<sup>+</sup> increased and aw decreased, and on the contrary, an increase was seen when Mg<sup>2+</sup> increased. More robust statistics are required in the text, showing the R<sup>2</sup> or the associated p-value in the text.

Figure 8 seems illustrative to me but confusing in its interpretation, and I also suggest increasing the size of the axes and the legend. It is not entirely clear what the abundance profiles are in the figure. The axes are aw and Mg, but is the abundance of the taxa relative? absolute? Is it the area of the violin plot?

In terms of changes in community composition, the results could include changes in relative abundance like the Kruskal–Wallis test or ANOVA to determine if the changes of certain taxa are significant between BH and P.

#### Discussion

The discussion is clear, with great coverage of the literature to achieve a deeper understanding of the results.

#### Perhaps

I would recommend a final paragraph with a projection for future work or approaches.

The first paragraph, line 7 of the discussion in The microbial community section, presents an unreferenced sentence (called REF).

It would be interesting to compare the composition of the ponds with some lagoons or lakes with high salt concentration in the Atacama Desert or those named in the introduction, to see if the microbial diversity is shared to some extent.

## Declarations

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