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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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Demargasso et al. ("The Limits of Life at Extremely Low Water Activity: Lithium-Concentration Ponds in a Solar Saltern (Salar de Atacama, Chile)") investigate the limits of life along a salinity gradient or a water activity gradient, respectively, in Li-concentration ponds. The authors found DNA of archaea in ponds with lower salinity, while the DNA of bacteria was present down to an aw of 0.2.

This is an interesting piece of work that is informative not only for the extremophile community, and it is absolutely worth being published. However, it needs some revision beforehand.

The manuscript is well-written, but the readability could be improved: There are often short sentences stringed together that seem rather unrelated. Its composition needs some revision; the structure of the manuscript could be clearer in many parts. For instance, the results section contains parts that belong to the discussion, while the discussion contains information that should already be given in the introduction. The introduction should contain a core hypothesis or objective that is picked up again at the end of the discussion. In addition, there are some flaws that can be easily eliminated. For instance, abbreviations should only be introduced once and then used throughout the manuscript (the only exception is the abstract), sentences should not start with an abbreviation, present and past tense should not be mixed, etc.

Sometimes, the wording is not very scientific; for instance, "The presence of Burkholderiales at the lowest aw ponds was intriguing." - there is no context given. Why is it intriguing? I suggest moving this sentence with the respective explanation to the discussion section. Another example is "The first reaction any microbiologist will have..."

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The readability of the figures should be enhanced (larger font, not bold), and the design of the figures should be adjusted.

Some more specific comments:

Abstract

Overall, the abstract could be better structured. You report, for instance, that life at an aw of 0.61 is possible, name the respective organisms, and then repeat that some microorganisms are capable of growth at such a low water activity. It is also not clear why you use the term "by contrast" afterwards - which contrast do you want to emphasize, between high-sugar and high-salt substrates?

Introduction

- What is the difference between hypersaline environments and salt-rich environments? Do you mean the difference between NaCl-dominated environments and environments dominated by other types of salt? You could add here the information about chaotropic vs. kosmotropic (now in the discussion).
- As already suggested by Mauricio Latorre and Ignacio Ramos-Tapia, it would improve the manuscript if the introduction would follow a clear thread towards the hypothesis or the core objective. For instance, the sentence "Our purpose was to find..." is placed between the description of saline environments; it would be better to first describe the environments and their special properties and subsequently your approach and your hypothesis or objective.

Material and Methods

- The passage about possible contaminations (at the moment, only one short sentence) should be more elaborate. Are there any possible sources of contamination? What about sampling and transport?
- To meet scientific standards, I suggest adding at least basic information on the manufacturer to all used devices and products.
- The last sentence in the section 'Statistical analysis of microbial diversity' seems to belong somewhere else.

Results

- It might be an option to put the different approaches (and their comparison) for the determination of water activity in the methods section, as they represent not the main interest of this study.
- Figure 8 is highly informative but should be designed to be easier to comprehend. This is just an optional idea: Could the color gradient be replaced by a grid representing aw values? And it would be helpful to clearly indicate the gradient in the figure (arrow, etc.). Both panels should also have the

same size.

- Some of the figures should be moved from the main manuscript to the supplementary material.

- Figures 10 and 11 could also benefit from a clear indication of the aw gradient.

- The information on how enrichment cultures were obtained should be included in the methods section.

- What do you mean by "Currently, the P6 and Consortium enrichments are kept in artificial brine"?

Discussion

- The subheadline "Chemical composition along the gradient" does not really fit the content here. In general, I suggest changing the subheadlines in the discussion to more meaningful terms: "The microbial community" could be replaced by a headline that indicates the interesting results regarding the microbial community, etc.

- It is not necessary to repeat the processes in the ponds here (first paragraph of the discussion).

- Line 6 of the discussion: There is a reference that differs from other references.

- The basic information you name here should be rather transferred to the introduction section. The discussion should solely focus on the results of the presented work in context with previously published studies.

- Detailed information on deep-sea basins should also be transferred to the introduction section. Important with regard to the discussion is only the fact that it was deemed to be sterile.

- In the first paragraph of "The microbial community" section, a reference is missing (indicated by "REF").

Declarations

Potential competing interests: No potential competing interests to declare.