

## Extremophiles from Tirez and Peña Hueca: Implications for exploring habitability of Mars and Europa

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### Abstract

Tirez and Peña Hueca are endorheic hypersaline lagoons of Villacañas, in the province of Toledo, Western La Mancha, Spain. The chloride and sulphate rich environment of endoreic origin of these lagoons potentially serve as Planetary field analogue sites for Martian chloride deposits and oceans of Europa. Extreme halophiles are candidate exophiles to study the adaptation and response of microbial growth in space like conditions [1]. Hence, the objective of the present investigation was to isolate extremophilic halophiles that thrive in brines of Peña Hueca rich in Mg-Na-SO<sub>4</sub>-Cl, epsomite and hexahydrate and study its implications for exploring habitability of Mars and Europa.

### 1. Introduction

Laguna de Peña Hueca is a unique hypersaline ion rich system in La Mancha, central Spain. The salt rich environment of endoreic origin also provides a good analogue for Martian chloride deposits and Europa brines. Studying extremophilic biodiversity from Peña Hueca along with Tirez lake may provide useful insights in plausible exploration of habitability or quest for microbial life in Martian environments

### 2. Methodology and Observations

A visit to Tirez lake and Pena Hueca field site was conducted from 9 to 13 April 2018 under Europlanet Transnational Access (TA) (17-EPN3-030). Pena Hueca was a unique pink colored lagoon located at 653 m altitude, 39° 30' 56" N 3° 20' 21" W (UTM WGS84) with maximum depth of 40 cm. It was characterized by its bright pink colored water that had a thick layer of pink colored salt crust. Underlying the crust, a typical green mat was observed below which a black anoxic layer was observed. Samples and rocks were collected from the lagoon and analyzed for

physicochemical parameters like sodium, potassium, chloride, magnesium content [2].



Fig 1: Pink colored hypersaline Laguna de Peña Hueca.

Laguna de Peña Hueca had a salinity of 12.5 % with a sulphate content of 18.75g/L, magnesium content of 9.04 g/L and a pH range of 7.5-7.9. Extremely halophilic organisms were isolated from rocks collected from Peña Hueca using Sehgal and Gibbons medium [3] containing 1.5 M NaCl and 0.5 M MgSO<sub>4</sub>. The organism was identified using biochemicals and 16 S rRNA gene sequencing. The organism was exposed to high concentration of salinity, epsomite, sodium sulphate and perchlorate as described earlier [3,4] to explore its potential survival in Martian conditions.

### 3. Summary and Conclusions

Laguna de Peña Hueca is an interesting hypersaline ion rich system that can be used as a planetary field analogue site for Mars or Europa. The shallow stagnant lagoon has high salinity and sulphur content

that metabolically favors combined biogeochemical activities of Sulphur reducing bacteria, purple or green Sulphur bacteria and halophilic bacteria. A distinct gradation of microbial communities can be observed and the black layer indicated biological production of hydrogen sulphide that causes precipitation in sediment. The sulphides are oxidized by photosynthetic bacteria that form a typical green mat and represent photosynthetic bacterial and cyanobacterial communities. The system forms a typical habitat called “Sulfuretum” that is Sulphur rich due to biological activities and geological deposits. The rocks present in these sulphur rich brines serve as potential zones for harboring endolithic bacterial communities at the water:rock interface. The survival of micro-organism in high sulphated saline system is imperative to understand the underlying implications of sulphate in microbial growth. Extremely halophilic bacteria *PLR-1* was isolated from a pink rock submerged in the hypersaline sulphate rich brine of Peña Hueca. The extremophile was identified as *Halomonas gomseomensis* PLR-1 and was found to be extremely resistant to high concentration of salinity (upto 4.5 M sodium chloride), epsomite concentration (upto 0.5 M Magnesium sulphate, perchlorate (upto 1M sodium perchlorate) and sulphate (upto 1 M sodium sulphate). The tolerance of this extremophile to high concentration of epsomite, salinity, sulphate and perchlorate demonstrates its ability of growth in Martian soils. The current study highlights the resilience of extremophiles from Planetary field analogues to Martian conditions and its implications and concerns in Planetary protection as these extremophiles may contaminate spacecraft and can thrive in Martian conditions.

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