

Gómez-Villegas P., Guerrero JL., Pérez-Rodríguez M., Bolívar JP., Morillo A., Vígara J., León R. (2022) Exploring the microbial community inhabiting the phosphogypsum stacks of Huelva (SW, Spain) by a high throughput 16S/18S rDNA sequencing approach. *Aquatic Toxicology* 245: 106103 Doi: 10.1016/j.aquatox.2022.106103.

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Abstract

Around 100 Mt of phosphogypsum (PG) have been deposited in large stacks on the salt marshes of the Tinto River estuary in Huelva (SW Spain), covering about 1000 ha. These stacks contain extremely acidic water ($\text{pH} < 2$) with high concentrations of pollutants which can cause emissions into their surroundings, generating important environmental concerns. Despite many chemical, geological or hydrological studies have been conducted to characterize the PG stacks of Huelva, the microbial community inhabiting this extreme environment remains unexplored. Using a 16S/18S-rRNA-high throughput sequencing approach, we have uncovered the main taxonomic groups able to live in the acidic metal-contaminated water, which is in direct contact with the PG, demonstrating for the first time the existence of a huge diversity of microbial species in these extreme conditions. In addition, the physicochemical characteristics of the water sampled have been analyzed. These studies have revealed that the most abundant bacteria found in two different leachate samples of the PG stacks belong to the genera *Acidiphilium*, *Pseudomonas*, *Leptosprillum*, *Acidithrix*, or *Acidithiobacillus*, typically found in acid mine drainage (AMD) environments, which in total represent around 50% of the total bacterial community. Biodiversity of eukaryotes in PG water is lower than that of prokaryotes, especially in the water collected from the perimeter channel that surrounds the PG stacks, where the pH reaches a value of 1.5 and the activity concentrations exceed 300 Bq L^{-1} for ^{238}U or 20 Bq L^{-1} for ^{210}Po , values which are from four to five orders of magnitude higher than those usually found in unperturbed surface waters. Even so, an unexpected diversity of algae, fungi, and ciliates have been found in the PG stacks of Huelva, where chlorophyte microalgae and basidiomycetes fungi are the most abundant eukaryotes. Additional bioinformatics tools have been used to perform a functional analysis and predict the most common metabolic pathways in the PG microbiota. The obtained data indicate that the extreme conditions of these PG stacks hide an unexpected microbial diversity, which can play an important role in the dynamics of the contaminating compounds of the PG and provide new strains with unique biotechnological applications.

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