# The importance of native microbial communities in the sustainability of vegetation for the phytomanagement of semiarid mine tailings

# Importancia de las comunidades microbianas autóctonas en la sostenibilidad de la vegetación para el fitomanejo de zonas mineras semi-áridas

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***Abstract***

**This proposal of PhD thesis will focus on proposing suitable scientific and technical alternatives for the phytomanagment of metal(loid) enriched mine tailings in the Sierra of Cartagena-La Unión (SE Spain). Thus, a strategy for the environmental restoration at middle-long term will be implemented. The main goal of the project is to evaluate the effects of the addition of organic amendments (combination compost/biochar) and/or of plant growth on the structure of native microbial communities and the improvement of edaphic parameters for the phytomanagement of mine tailings in semiarid areas. The enhancement of soil functionality will be assessed attending to microbiological changes, including the structure of microbial populations and the behaviour of pioneer plant species (plant biomass, metal uptake).**

**Keywords**: metal(loid); microorganisms; ecological succession; soil amendments.

***Resumen***

**La presente propuesta de tesis doctoral busca aportar soluciones científico-técnicas para el fitomanejo de residuos mineros en la Sierra de Cartagena-La Unión, como parte de una estrategia de restauración ambiental sostenible a largo plazo. El objetivo general de la tesis doctoral es valorar en qué medida la adición de enmiendas orgánicas (combinación compost/biochar) y/o establecimiento de plantas pueden afectar a la estructura de las comunidades microbianas autóctonas y desencadenar procesos de evolución edáfica que redunden en la mejora de la funcionalidad del suelo desde el punto de vista microbiológico, incidiendo en aspectos relacionados con los cambios en la microbiología del suelo y el comportamiento de especies vegetales autóctonas (biomasa, acumulación de metales) dentro del contexto del fitomanejo de residuos mineros en zonas semiáridas.**

**Palabras clave:** metales; microorganismos; sucesión ecológica; enmiendas edáficas.

## 1. INTRODUCTION

Mine tailings cause the main environmental impacts in former metal ore mining areas [1]. The extreme physico-chemical properties of tailings such as contrasting pH values, high salinity, high metal(loid) concentrations, low fertility, etc. lead to the occurrence of bare surfaces prompt for erosion [1]. Previous findings in semiarid tailings have highlighted the use of pioneer plant species combined with amendments(phytomanagement by phytostabilization) as the best tool to perform their surface stabilization [2, 3]. The goal in these sites should focus in improving soil functionality and the sustainability of the nutrient biogeochemical cycles over time, which could lead to generate a self-sustaining vegetation cover [4]. In this process, native microbial communities play an important role by supporting the cycling of nutrients, preventing plants from metal phytotoxicity or promoting plant ecological succession [5].

Based on these previous issues, and taking as a study site a former tailings disposal area of the semiarid Cartagena-La Unión Mining District, the following goals are proposed: 1) to evaluate the changes of the native microbial community generated by the existence of edaphic gradients in mine tailings; 2) to evaluate the effects of the addition of organic amendments and / or plant growth in the structure of the native microbial community; 3) to evaluate the sustainability of the microbial communities present in the tailings and their capacity to carry out the transition from systems conditioned by the addition of organic amendments to those sustained by their own leaf litter; 4) to evaluate the effect of amendments on the behavior of the native plant species and its relation with the microbiology of the mine tailings.

## 2. MATERIAL AND METHODS

The PhD thesis is divided in two phases:

### 2.1 Field phase

In this part, a transect sampling will be performed from an unpolluted area (taken as a *control*) to the mine tailings pile's plateau, distinguishing several microenvironments for soil sampling (fig 1). In each microenvironment at least three composite samples will be taken for physico-chemical analyses (*e.g.* metal(loid) concentration, pH, electrical conductivity, total nitrogen, etc.) and for the characterization of the microbial DNA by isolation and amplification with specific primers to ITS (Internal Transcribed Spacer) or rRNA16S for fungi or prokaryotes, respectively. These results of PCR will be analyzed by massive sequencing and the sequences obtained will be studied by bioinformatics and statistical programs such as CANOCO and SPSS.

### 2.2 Experimental phase

In this experiment the effect of the combination of amendments/plant establishment on microbial communities will be evaluated. Soil from tailings of the field phase will be used. Tailings soil will be mixed with 4% compost, 4% biochar and with a mixture of 4% compost and 4% biochar, obtaining three treatments along with a series of bulk tailings treatment (no amended). Soil from each pot (12 repetitions by each treatment) will be characterized at the beginning as it was performed for field samples (paragraph 2.1). This experiment will be developed in a controlled growth chamber (temperature, light). Pots will be maintained at half of field capacity and the physico-chemical and microbial DNA will be monitored periodically. At the sixth month, each treatment (12 pots) will be sub-divided in three groups (4 pots for each one): a group with no plant, another group will be implanted with seeds of *Piptatherum miliaceum* (a pioneer plant species from the tailings) and, in the last one, leaf litter of *P. miliaceum* will be incorporated. The experiment will continue for 12 more months and characterization of plants (biomass and ionomic analyses), edaphic parameters and microbial DNA will be carried out at the end.

## 3. EXPECTED RESULTS

Preliminary results have been obtained at the field sampling phase. All soil samples showed alkaline pH (7.2-7.9), even so, the samples at the tailings showed deficient conditions for plant growth: low organic carbon (~0.4%) and total nitrogen (~0.05%), high electrical conductivity (2-3 dS $m^{-1}$ in 1:5 soil:water extract) and high metal(loid) concentrations (*e.g.* 6000 mg $kg^{-1}$Pb; 9000 mg $kg^{-1}$ Zn). Areas closed to the tailings, (external border at fig.1) showed similar metal(loid) concentrations than the tailings´ pile samples. Although the most abundance bacterial phyla were *Actinobacteria*, *Proteobacteria* and *Acidobacteria* in all sampling zones (more than 50% of total abundance) (Table 1), a shift in relative abundances occurred between areas. The phylum *Actinobacteria* showed higher relative abundance in the non-vegetated samples at the tailings (*Bulk* and *Internal Border*) than at the forest samples, which could be explained by its oligotrophic character [6]. In the case of the phylum *Proteobacteria,* tailings samples showed higher percentages of abundance than samples out of the tailings. This phylum has been shown to be metal tolerant and one of the most abundant phyla in metal enriched tailings worldwide [7]. Probably because of alkaline conditions, the phylum *Acidobacteria,* was not predominant at the tailings samples. This phylum usually appears as dominant in strongly acid tailing wastes worldwide [8]. Other bacterial phylum, such as *Planctomycetes*, *Bacteroidetes* and *Verrucomicrobia*, showed relatives abundances lower than 14%.

## 4. CONCLUSIONS

The initial data showed a closer relationship of the microbial population structure with the edaphic parameters than with the concentration of metals, indicating a tolerance behavior of some phylum for the latter. In addition, the changes in the microbial communities were also related to the presence or absence of vegetation. For instance, the phylum *Proteobacteria* seemed to play an important role in the early plant successional establishment at the tailings, which was at the same time facilitated by the absence of acidic conditions.

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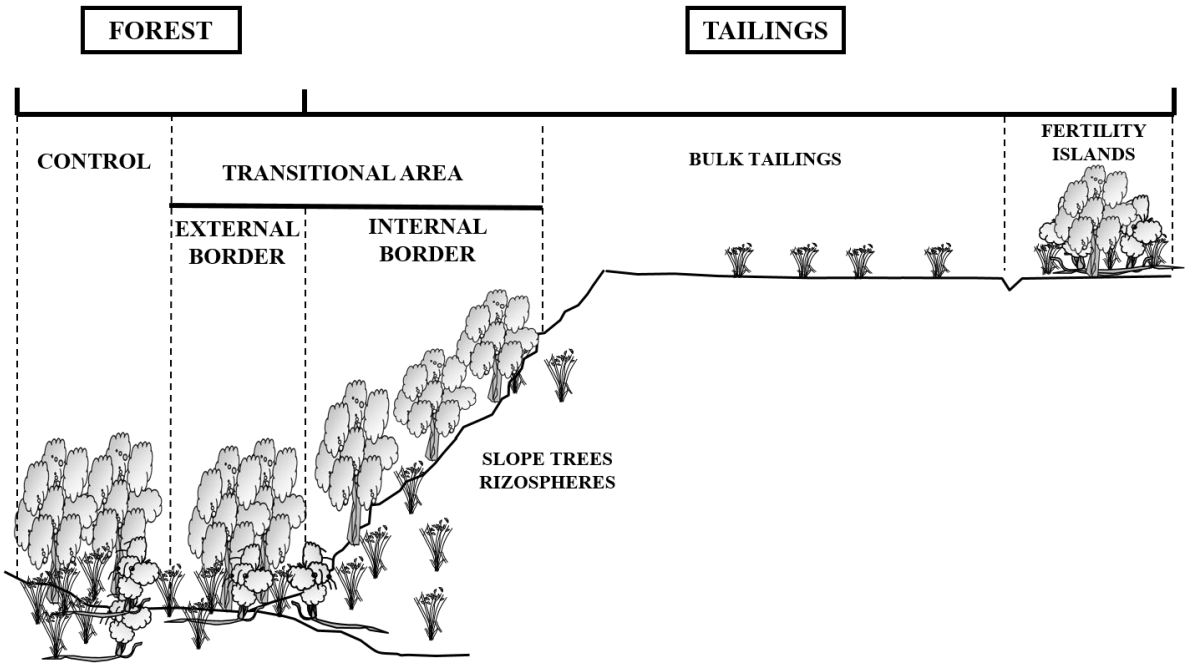


**Figure 1.** Scheme of the sampling transect.

**Table 1.** Abundance relative for transect microenvironments. Data are average ± standard error. Different letters among columns of the same row indicate significant differences (ANOVA with Tukey`s test, p<0.05). Number of replicates are 3 or 6.

| Bacterial Phylum | Mean Relative Abundance (%) | | | | | |
|---|---|---|---|---|---|---|
| | Forest | | Tailing | | | |
| | Control | External Border | Internal Border | Slope Trees Rhizospheres | Bulk Tailing | Fertility Island |
| ***Acidobacteria*** | 15.8 ± 1.0 b | 17.0 ± 0.2 b | 10.1 ± 1.2 a | 10.9 ± 0.9 a | 8.9 ± 0.6 a | 10.6 ± 0.9 a |
| ***Actinobacteria*** | 23.4 ± 1.3 ab | 17.3 ± 0.1 a | 38.6 ± 3.0 cd | 26.7 ± 1.3 b | 42.9 ± 1.9 d | 29.8 ± 3.6 bc |
| ***Bacteroidetes*** | 7.8 ± 0.6 bc | 11.3 ± 1.1 c | 2.6 ± 0.6 a | 10.8 ± 1.4 c | 3.6 ± 0.3 ab | 8.3 ± 0.5 c |
| ***Candidatus Saccharibacteria*** | 1.6 ± 0.1 ab | 3.1 ± 0.3 b | 0.9 ± 0.1 a | 5.4 ± 0.3 c | 2.9 ± 0.6 b | 3.3 ± 0.4 b |
| ***Chloroflexi*** | 2.0 ± 0.2 ab | 1.2 ± <0.1 a | 2.4 ± 0.3 b | 1.7 ± 0.2 ab | 1.6 ± 0.1 ab | 2.3 ± 0.4 b |
| ***Gemmatimonadetes*** | 3.3 ± 0.5 a | 2.9 ± 0.1 a | 8.8 ± 0.4 b | 3.9 ± 0.5 a | 3.6 ± 1.1 a | 4.3 ± 0.6 a |
| ***Planctomycetes*** | 13.2 ± 0.7 c | 12.8 ± 1.0 bc | 5.3 ± 1.2 a | 6.5 ± 1.1 a | 4.5 ± 0.3 a | 8.7 ± 1.4 ab |
| ***Proteobacteria*** | 16.8 ± 0.2 a | 19.7 ± 0.3 a | 28.7 ± 0.6 b | 26.1 ± 1.4 b | 29.0 ± 1.3 b | 26.7 ± 1.9 b |
| ***Verrucomicrobia*** | 6.4 ± 0.4 c | 6.1 ± 0.4 c | 1.0 ± 0.3 a | 4.0 ± 0.5 b | 1.3 ± 0.2 a | 2.7 ± 0.2 ab |
| **Others** | 9.8 ± 0.6 b | 8.6 ± 0.3 b | 1.8 ± 0.2 a | 4.0 ± 0.8 a | 1.7 ± 0.5 a | 3.3 ± 0.2 a |