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# CONTAMINATED SITES 2022

SENEC, SLOVAK REPUBLIC, 12 – 14 OCTOBER 2022

*The activity has been implemented within the framework of national project  
**Information and providing advice on improving the quality of environment in Slovakia.**  
The project is cofinanced by Cohesion Fund of the EU under Operational programme Quality of Environment.*

# Study of the vegetation cover of an environment contaminated by heavy metals sulphides: Strategies and specific distribution

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# FRAMEWORK: SULFIDE MINERAL POLLUTION

To gather knowledge about:

Soil microbiological activity

Plant physiology of resilience

Mine lands reclamation



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Research paper

## Microbial diversity and activity assessment in a 100-year-old lead mine

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ARTICLE INFO

ABSTRACT

Mining activities frequently leave a legacy of oxides that remain in the area for long periods causing the pollution of surroundings. We studied on a 100-year-old mine, the behavior of potentially toxic elements (PTEs) and their geochemical impact on activity and diversity of microorganisms. The PTEs contamination increment allowed the classification of the materials as highly (reference and contaminated samples) and very highly polluted (illegal spill of olive mill wastes (OMW), tailings, and dumps). OMW presented the lowest enzymatic activities while tailings and dumps had low dehydrogenase and arylsulfatase activities. All the activity indices studied were negatively impacted in dumps. Tailings had lower Chl *a* and PS whole tree values as compared to those of reference samples.  $\beta$ -diversity analysis showed similar bacterial community composition for reference and contaminated samples, significantly differing from that of tailings and dumps. The relative abundance of Gemmatimonadetes, Bacteroidetes, and Verrucomicrobia was lower in OMW, tailings, and dumps as compared to reference samples. Fifty-seven operational taxonomic units were selected as responsible for the changes observed between samples. This study highlights that assessing the relationship between physicochemical properties and microbial diversity and activity gives clues about ongoing regulating processes that can be helpful for stakeholders to define an appropriate management strategy.

## 1. Introduction

Mining activities are found all over the world because they provide access to mineral resources that fuel various industrial activities in both developed and in developing countries. Although recognized of great importance for the world gross domestic product, mining operations are often viewed as an important source of pollution with negative impact on the environment. During the process of mineral extraction and preparation, large amounts of one wastes and debris are commonly accumulated in the vicinity of the mining operation site. These materials are essentially fractured rocks and soil devoid of vegetation, characterized by high concentrations of heavy metals and metalloids. Consequently, the environment is drastically transformed in highly polluted barren areas (Martín-Delgado et al., 2015; Sanchez-Gonzalez et al., 2019), which can be toxic to human health and soil life, including plants and microorganisms (Giller et al., 1998;

Nagajyoti et al., 2010; Yrjanheikki et al., 2012). Furthermore, potentially toxic elements (PTEs) from these polluted areas can transfer to surrounding aquatic and terrestrial compartments via leaching or runoff (Liang and Thammara, 1996; Kikurova et al., 2018; Fernández-Martínez et al., 2019; Elmajed et al., 2020), disperse in the atmosphere (Naharro et al., 2018, 2020; Escribá et al., 2020), and indirectly contribute to pollutant dissemination. The recent interest in the reclamation of abandoned mining sites in arid and semiarid regions for agricultural purposes highlights the need to understand the biogeochemical processes contributing to soil health and fertility (Kislovskii and Petrovskii, 2002; Mendes et al., 2008; Fava et al., 2018; Higuera et al., 2018). Microorganisms such as bacteria and fungi are key players in soil ecosystem services. They are involved in multiple geochemical cycles, influence plant growth and contribute to climate regulation and soil restoration, among others (Van Der Heijden et al., 2008;

Abbreviations: ABS, Arylsulfatase enzyme activity; DBA, Dehydrogenase enzyme activity; EC, Electric conductivity; OMW, Olive-mill waste; OM, organic matter; PNA, Phosphoglucose isomerase activity; PHL, Potentially toxic elements; PGL,  $\beta$ -glucosidase enzyme activity.  
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https://doi.org/10.1016/j.jhazmat.2020.124618  
Received 19 August 2020; Received in revised form 9 October 2020; Accepted 14 November 2020  
Available online 18 November 2020  
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## Deciphering lead tolerance mechanisms in a population of the plant species *Biscutella auriculata* L. from a mining area: Accumulation strategies and antioxidant defenses

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HIGHLIGHTS

- *Biscutella auriculata* tolerates high concentration of Pb without toxic symptoms being observed.
- Pb is mainly sequestered by PC2 and accumulated in the root cell wall and the vacuoles.
- Differential activation of antioxidant defenses was induced by Pb in leaves and roots.

GRAPHICAL ABSTRACT



ARTICLE INFO

ABSTRACT

**Article history:**  
Received 6 May 2020  
Revised in revised form 6 July 2020  
Accepted 13 July 2020  
Available online 20 July 2020  
Handling Editor: T. Cuijck

**Keywords:**  
Phytoremediation  
Oxidative stress  
ROS  
Phytocatalysis  
*Biscutella auriculata*

The uptake and distribution of Pb and the mechanisms involved in the metal tolerance have been investigated in a mine population of *Biscutella auriculata*. Seedlings were exposed to 125  $\mu$ M Pb(NO<sub>3</sub>)<sub>2</sub> for 15 days under semi-hydroponic conditions. The results showed an increase in the size of Pb-treated seedlings and symptoms of toxicity were not observed. ICP-MS analyses showed that Pb accumulation was restricted to root tissue. Imaging of Pb accumulation by diffraction histochemistry revealed the presence of the metal in vacuoles and cell wall in root cells. The accumulation of Pb in vacuoles could be stimulated by an increase in phytochelatin PC2 content. Pb did not promote oxidative damage and this is probably due to the increase of antioxidant defenses. In the leaves, Pb produced a significant increase in superoxide dismutase activity, while in roots an increase in catalase and components of the Foyer-Halliwel-Asada cycle were observed. The results indicated that *Biscutella auriculata* has a high capacity to tolerate Pb and this is mainly due to a very efficient mechanism to sequester the metal in roots and a capacity to avoid oxidative stress. This species could therefore be very useful for phytostabilization and reclamation of areas contaminated with Pb.

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Abbreviations: APS, Ascorbate peroxidase enzyme; AAA, Ascorbate; CAT, Catalase; DBAR, Dihydroxyacetone reductase; G6P, Glycolate oxidase; GR, Glutathione reductase; GRX, Glutathione S-transferase; GSH, Glutathione; S-transferase; MDR, Malonaldehyde; MDRAR, Malonaldehyde-oxaloacetate reductase; NADP-G6PDH, NADP-dependent 6-phosphate dehydrogenase; NADP-IRI, NADP-dependent isocitrate dehydrogenase; NADP-ME, NADP-dependent malate dehydrogenase; NO, Nitric oxide; PC, Phytochelatin; POD, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase.  
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https://doi.org/10.1016/j.chemosphere.2020.127271  
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## Abandoned Mine Lands Reclamation by Plant Remediation Technologies

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**Abstract:** Abandoned mine lands (AMLs) which are considered some of the most dangerous anthropogenic activities in the world, are a source of hazards relating to potentially toxic elements (PTEs). Traditional reclamation techniques, which are expensive, time-consuming and not well accepted by the general public, cannot be used on a large scale. However, plant-based techniques have gained acceptance as an environmentally friendly alternative over the last 20 years. Plants can be used in AMLs for PTE phytoextraction, phytostabilization, and phytovolatilization. We reviewed these phytoremediation techniques, paying particular attention to the selection of appropriate plants in each case. In order to assess the suitability of plants for phytoremediation purposes, the accumulation capacity and tolerance mechanisms of PTEs was described. We also compiled a collection of interesting actual examples of AML phytoremediation. On-site studies have shown positive results in terms of soil quality improvement, reduced PTE bioavailability, and increased biodiversity. However, phytoremediation strategies need to better characterize potential plant candidates in order to improve PTE extraction and to reduce the negative impact on AMLs.

**Keywords:** phytoremediation; phytostabilization; phytovolatilization; phytoextraction; abandoned mine lands; heavy metals; reclamation; oxidative stress; accumulation

## 1. Introduction

In recent decades, many countries have realized that abandoned metal mining operations greatly contribute to environmental degradation [1]. Metal mining radically transforms the natural environment, causing modifications to the land, as well as increased accumulation of potentially toxic elements (PTEs) in the ecosystems, which seriously undermines soil health and the viability of all living organisms [2]. The principal problem is caused by mining activity carried out in the past using inefficient technology in the absence of environmental protection regulations, leading to the accumulation of mining waste which is now a source of PTEs [3]. Regarding their role in biological systems, PTEs can be divided into essential and non-essential categories. Despite being required by living organisms for physiological and biochemical functions, at high concentrations, essential PTEs, such as copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn), can have a toxic environmental impact. On the other hand, living organisms do not require non-essential PTEs such as Pb, mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr) and antimony (Sb) [4]. Large concentrations of PTEs present in abandoned mine waste can

Sustainability 2021, 13, 6530. https://doi.org/10.3390/su13106530

www.mdpi.com/journal/sustainability

# San Quintín minesite

## What is it?

- An 50 years long abandoned mine land with different disturbed substrates composed by regolith materials from metal-sulfide mine exploitation.

## What do we have?

- A mosaic of different substrates naturally colonized by specialist plants, tough enough to endure the constrains imposed by the environment
- A perfect place to study the relationship between the plant cover and the substrate and soil development

## What are the key factors?

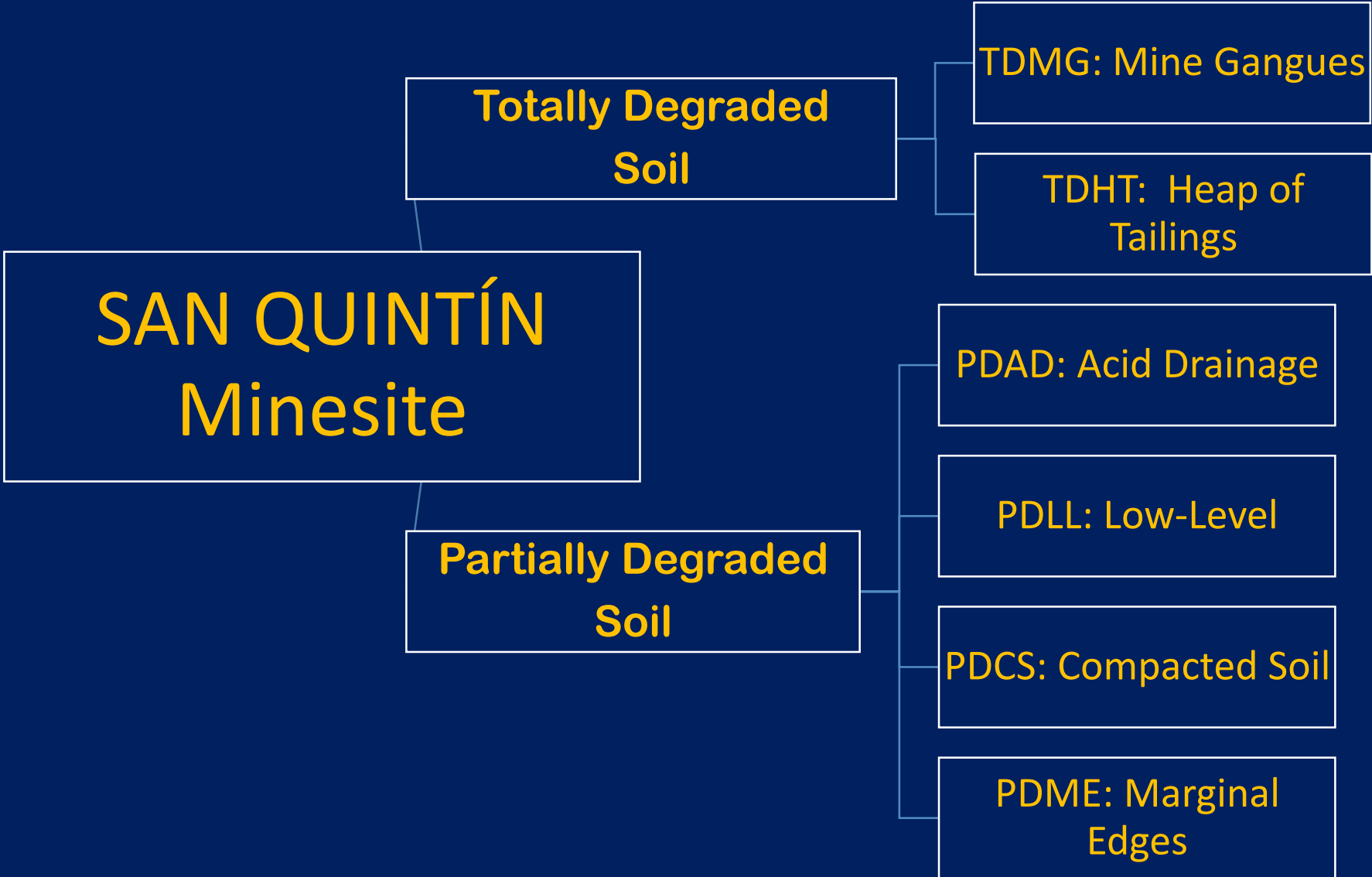
- A rainfall regime of about 400 mm per year
- A 5 months long-dry-hot summer
- A regolith substrate of sulfide minerals rich in heavy metals

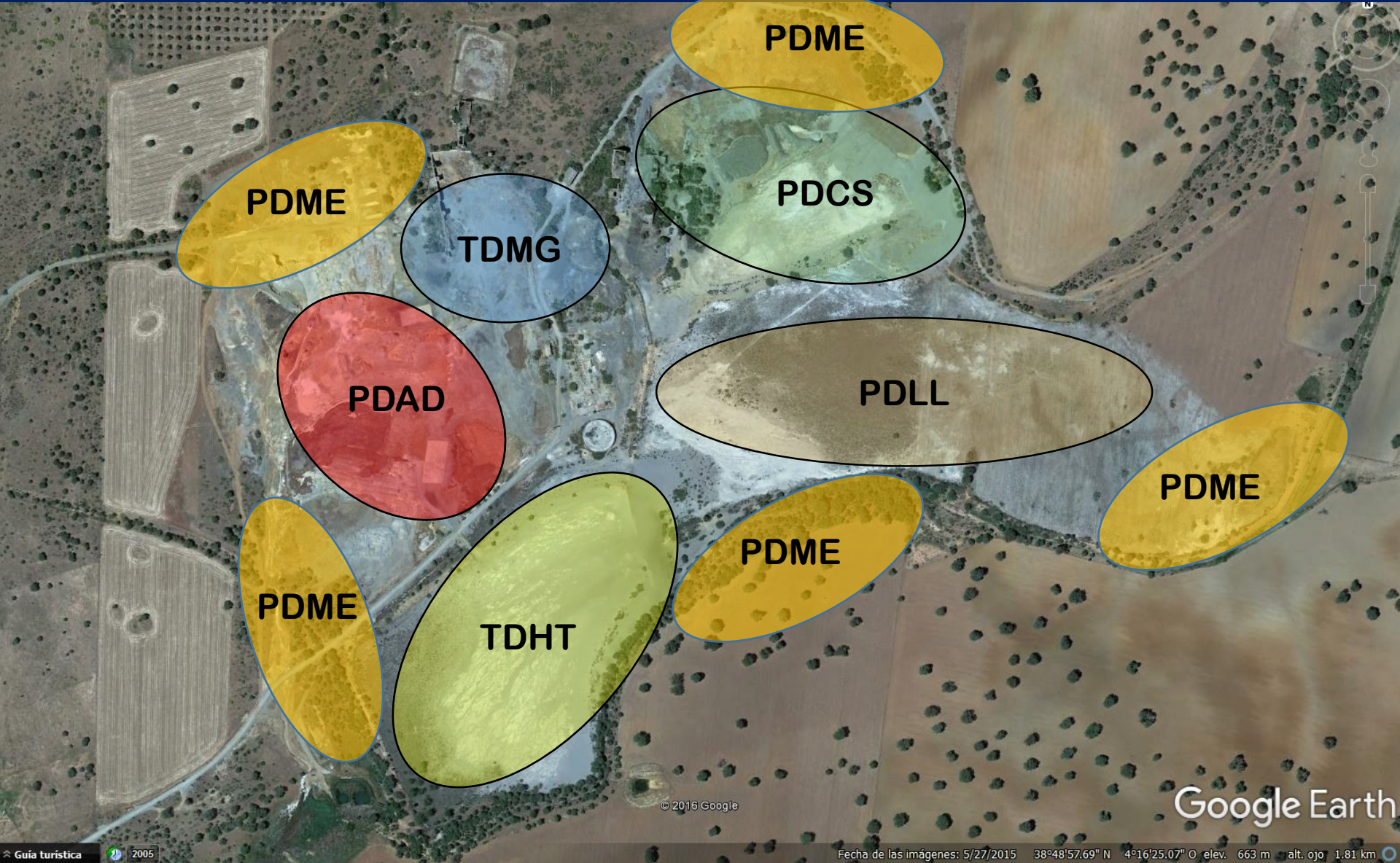
## What do we want?

To gather knowledge about:

- the patterns of natural colonization,
- the patterns of soil development
- the plant physiology behind the resilience to these habitats







**PDME**

**TDMG**

**PDCS**

**PDME**

**PDAD**

**PDLL**

**PDME**

**PDME**

**TDHT**

**PDME**

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# TDMG Mine Gangues





# TDMG Mine Gangues



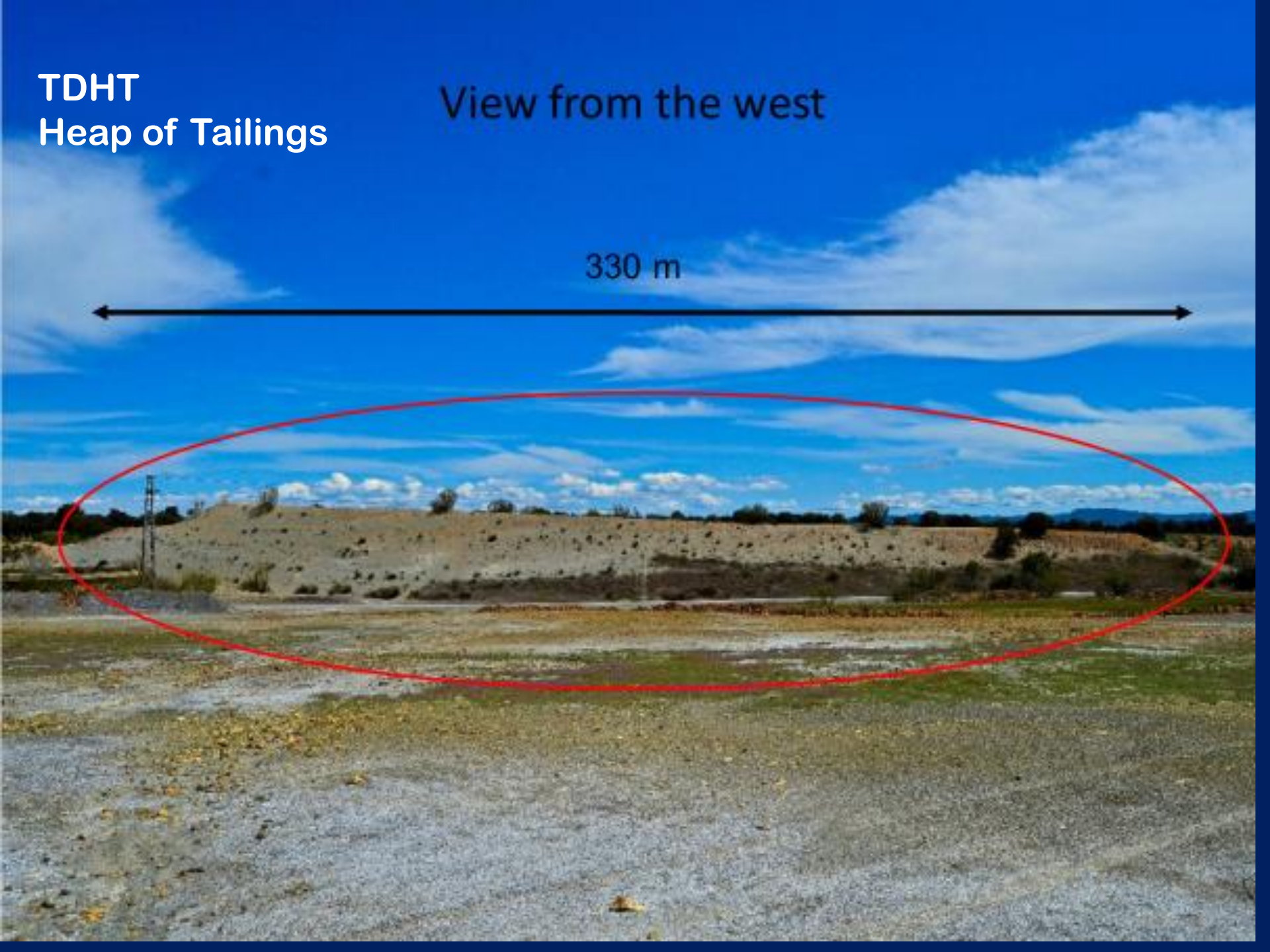
**TDHT  
Heap of Tailings**



**TDHT**  
**Heap of Tailings**

View from the west

330 m



*Scrophularia  
canina*

*Piptatherum  
milliaceum*





# PDLL: Low Level zones









# PDAD: Partially Degraded Acid Drainage zone







# PDCS: Compacted Soil zone











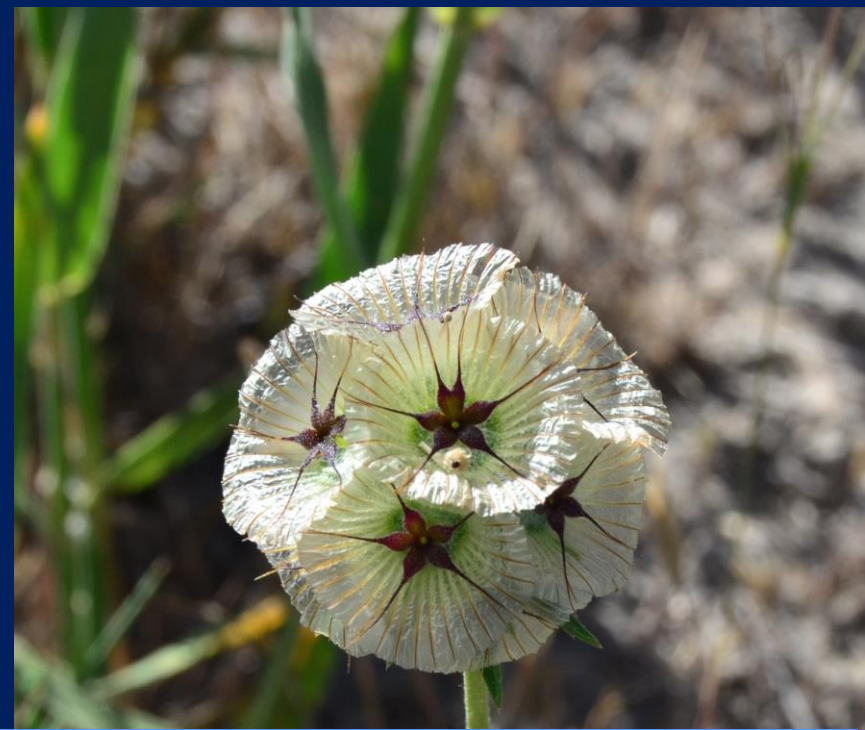


# PDME: Marginal Edges









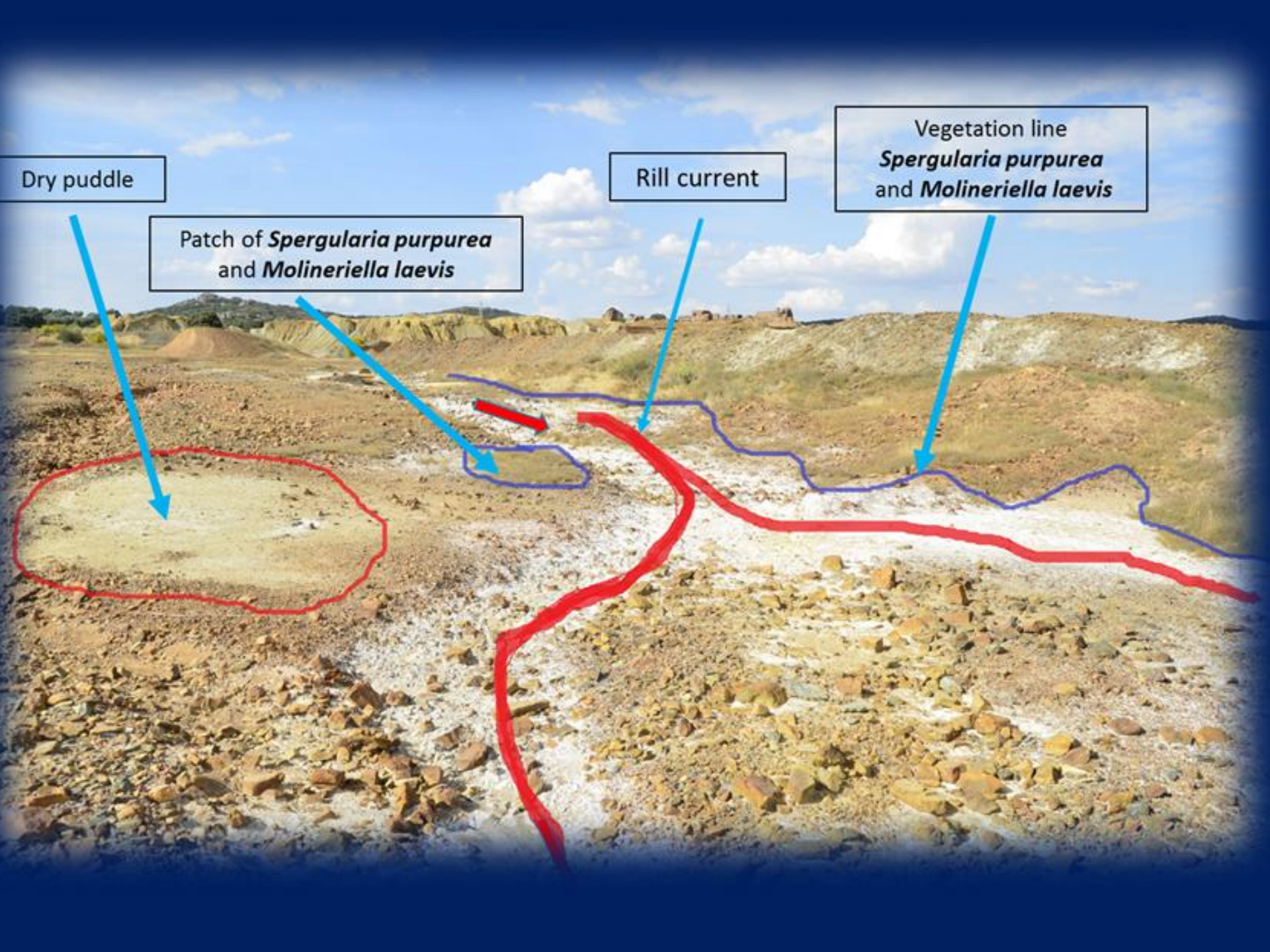
Two peculiarities about collaboration strategies:

*Spergularia purpurea and Molineriella laevis*

*Phragmites australis and Retama sphaerocarpa*

***Spergularia purpurea* and  
*Molineriella laevis* association**



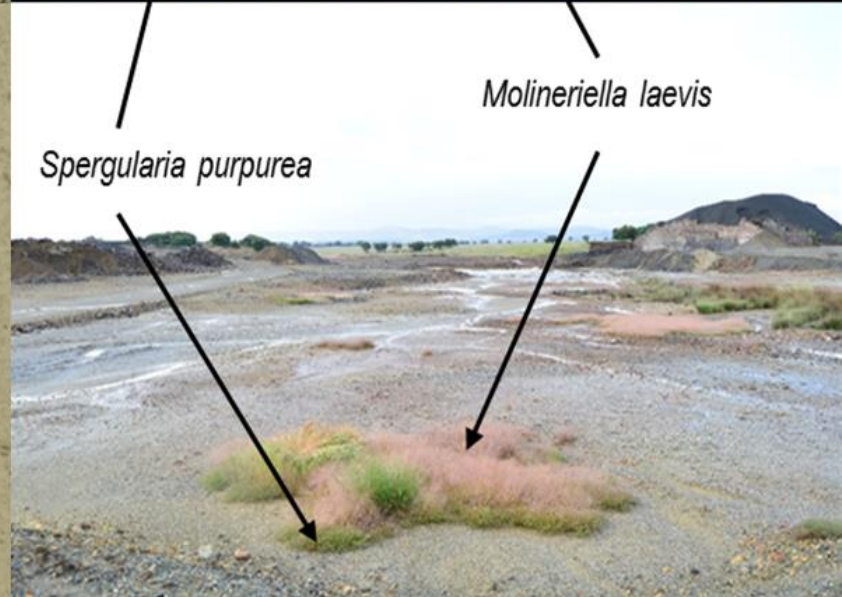
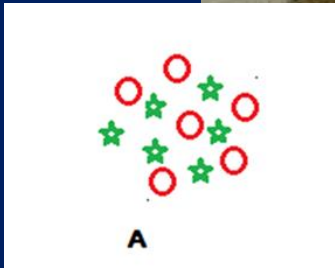
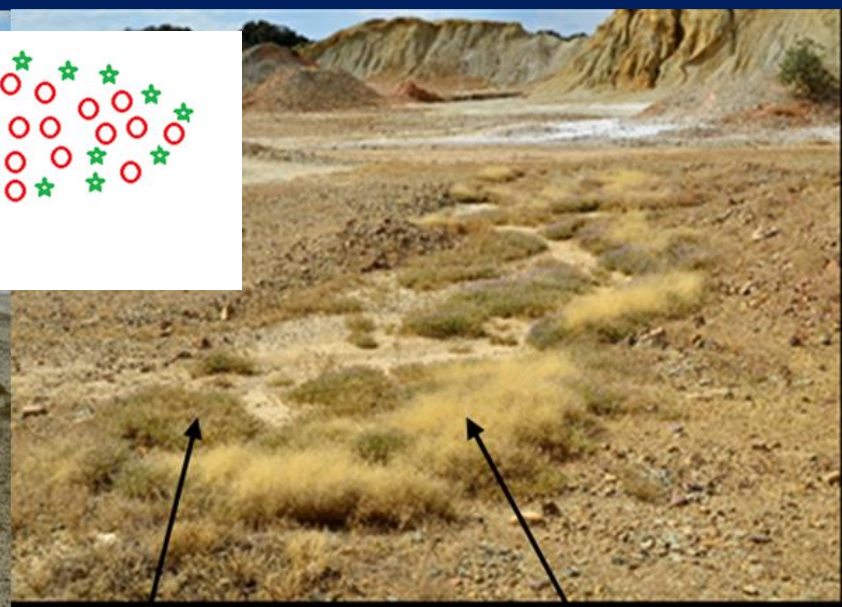
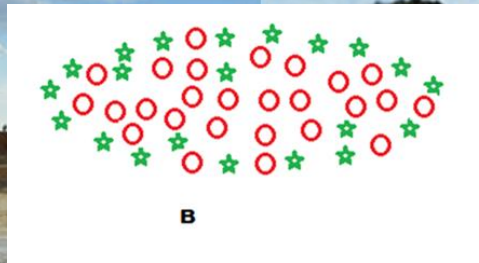


Dry puddle

Patch of *Spargularia purpurea*  
and *Molineriella laevis*

Rill current

Vegetation line  
*Spargularia purpurea*  
and *Molineriella laevis*

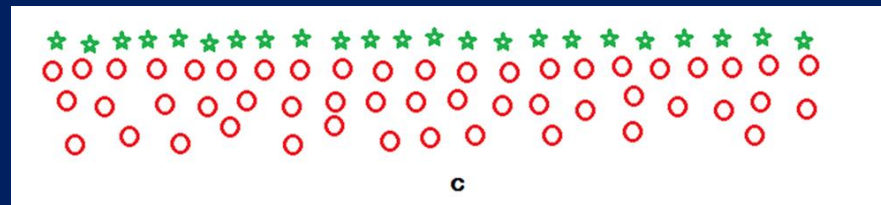


*Spergularia purpurea*

*Molineriella laevis*

★ *Spergularia purpurea*

○ *Molineriella laevis*











*Phragmites australis* / *Retama sphaerocarpa*  
collaboration















**MANY THANKS**