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# Plant-microorganism symbiosis: biological levers for the phytostabilisation of mine tailings

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# PHYTOSTABILIZATION

Combinations of soil technologies  
and

Plant / microbial species adapted  
to soil contaminated by Trace Metal Elements (TMEs)

Objectives: Soil stabilization

- Improve soil properties
- *In situ* containment : Implementation of a dense and perennial vegetation cover.



Soil improvment



Adapted plant and microorganisms

# Site of study: mine of Les Avinières, St Laurent-le-Minier (France)

40 km north of Montpellier

Opened in 1872 and closed in 1914

No redevelopment



Decantation areas



Mining spoil  
Strong wind and water erosion

	Zn	Pb	Cd	MO	Azote	Phosphore
	mg/kg			%	%	mg.kg <sup>-1</sup>
Decantation areas	116 022	37566	826	0,77	0,059	4,37
Mining spoil	83 808	59 775	313			
Not contaminated	233,54	59,61	1,71	2,65	0,14	23,38

# Les Avinières mining spoil

Several plant species adapted to high TMEs have been observed



*Noccaea caerulescens*



*Biscutella laevigata*



*Reseda lutea*



*Armeria arenaria*



*Iberis intermedia*



*Arenaria aggregata*

## Three of them are of major interest for the phytostabilisation of mining spoil



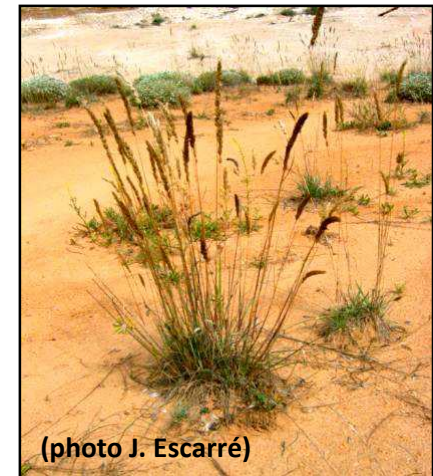
(photo J. Escarré)

*Festuca arvernensis*



(photo J.C. Cleyet-Marel)

*Anthyllis vulneraria*



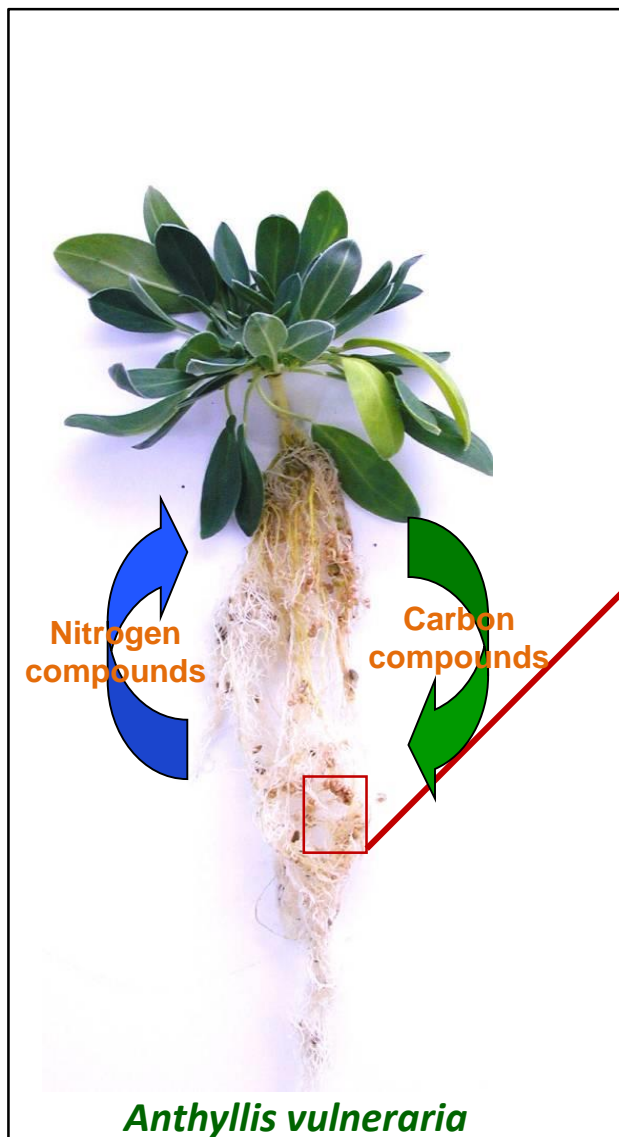
(photo J. Escarré)

*Koeleria vallesiana*

# Plant and microbial biodiversity

Biological  
Nitrogen  
Fixation  
 $N_2$

70% of plant nitrogen is  
derived from biological  
fixation  
(Mahieu et al., 2011)



New microbial species have  
been identified

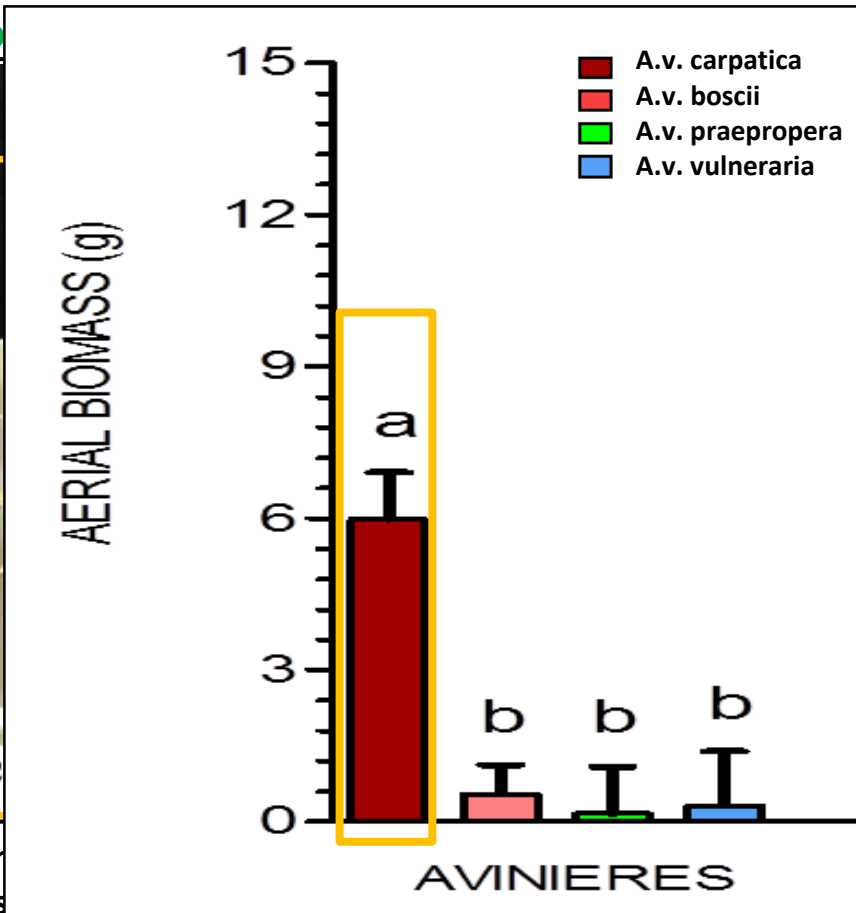
- ➡ *Mesorhizobium metallidurans*  
(Vidal et al., 2009)
- ➡ *Aminobacter anthyllidis*  
(Maynaud et al., 2012)

# Plant and microbial biodiversity

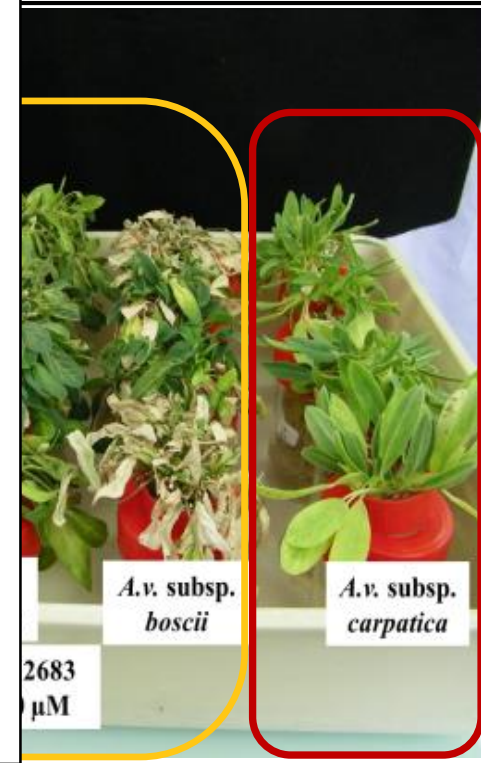
non-metallicolous



The different subspecies of *Anthracis* subspecies was



ous bacteria



containing 1000 μM Zn. Each *ous bacteria*.

- For the *carpatica* subspecies, high chlorophyll content was observed for both strains in the presence of Zn
- The metallicollous bacteria (*M. metallidurans* STM2683) has a protective effect on the host plant

(Soussou et al., 2013)

# *In situ* phytostabilization experiment

Implementation of the in situ experiment



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Planting with plant biotization  
(bacteria + fungi)

April 2012



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# *In situ* phytostabilization experiment

June 2012



1 year after installation



2 years after installation



Plot with non-biotized  
Anthyllis and Festuca



Plot with biotized Anthyllis  
and Festuca  
(bacteria + fungi)

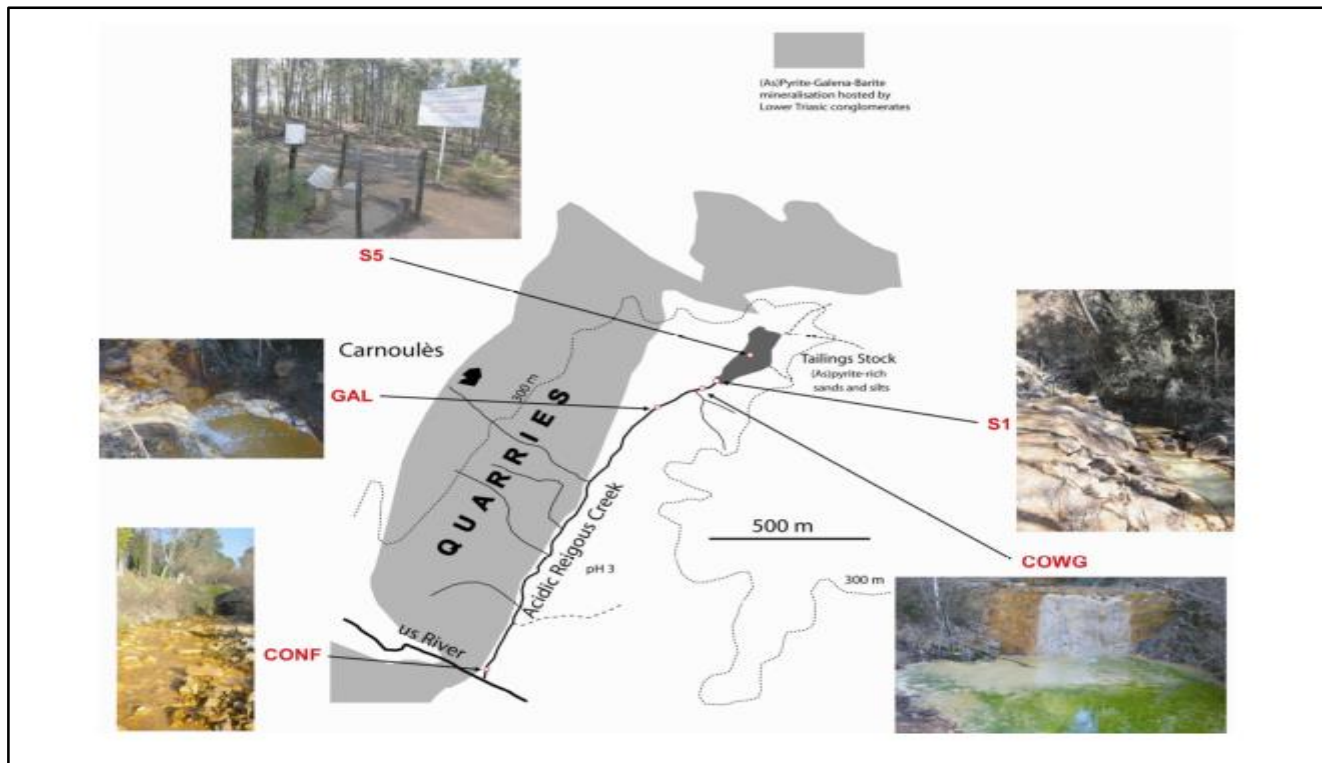
# Conclusions/ Part 1

**With SyMetal project, we demonstrated the importance of :**

- **Use of local plants and microbial species adapted to the TMEs constraint with complementary functionalities**
- **Phystabilization of the mine tailings to limit the pollution spread**
- ✓ **Control of the multiplication of plant species of interest: seed production**

# Saint Sébastien d'Aigrefeuille (30) Ancien site RECYCLEX

## Ancient mine of Carnoulès: Redevelopment study program and phytomanagement feasibility



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# Soil sampling for laboratory experiments



**Substrate with a **low pH (< 5)**, low organic matter (<0.5 %) and adverse physical characteristics for plant growth.**

**Metal concentrations (mg/kg) determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry):**

Zn	Pb	As
96592	10 000	1 800

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## Substrate preparation for laboratory experiments



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# Soil pH correction

## Limestone residues

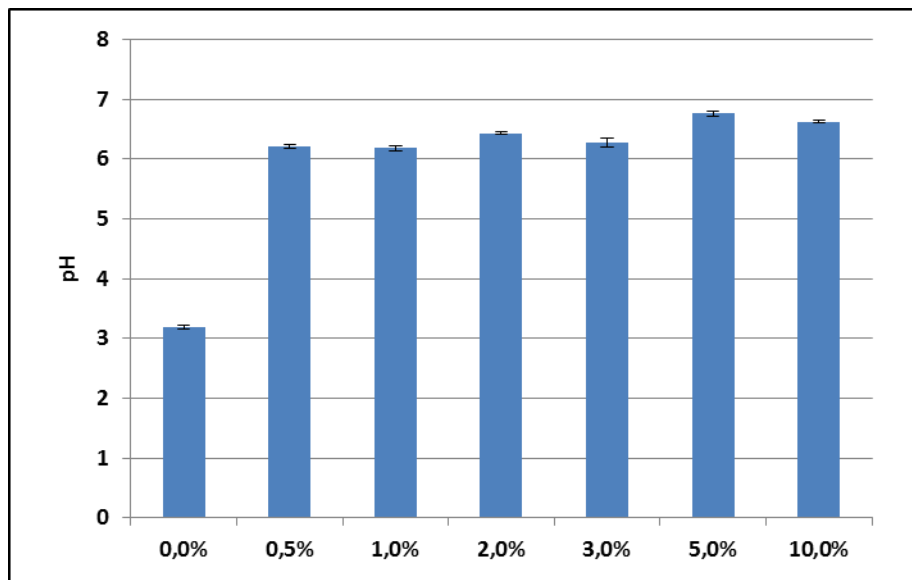


Use of varying grain size materials with high carbonate content :

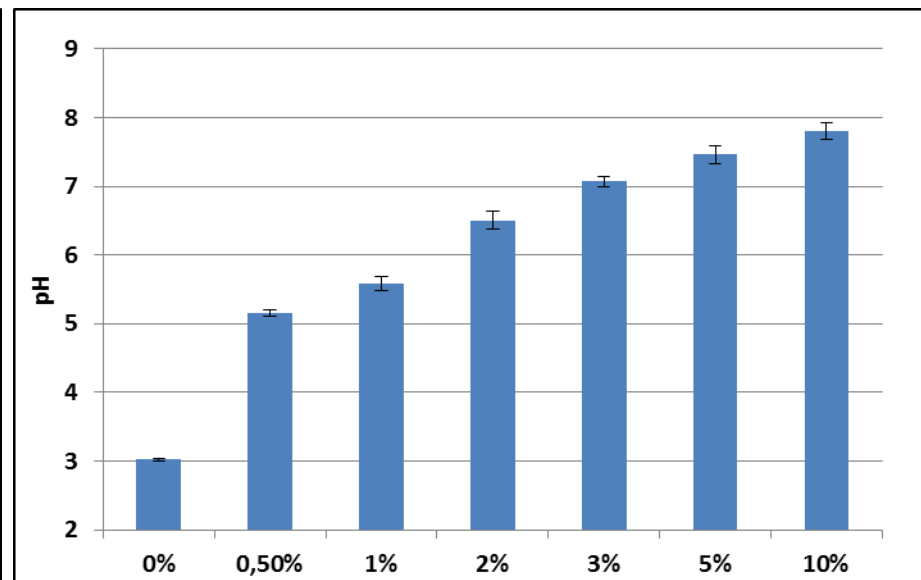
- fine fraction with an immediate effect on the pH
- coarse fraction with a progressive effect

# pH correction

## Limestone residues



## Compost



➤ The pH correction was achieved with the addition of limestone (from 0.5%) and compost (from 3%)

## Complex mixtures

Mining substrate (%)	100	98	97	95	93
Limestone residues	0	2	2	2	2
Compost	0	0	1	3	5

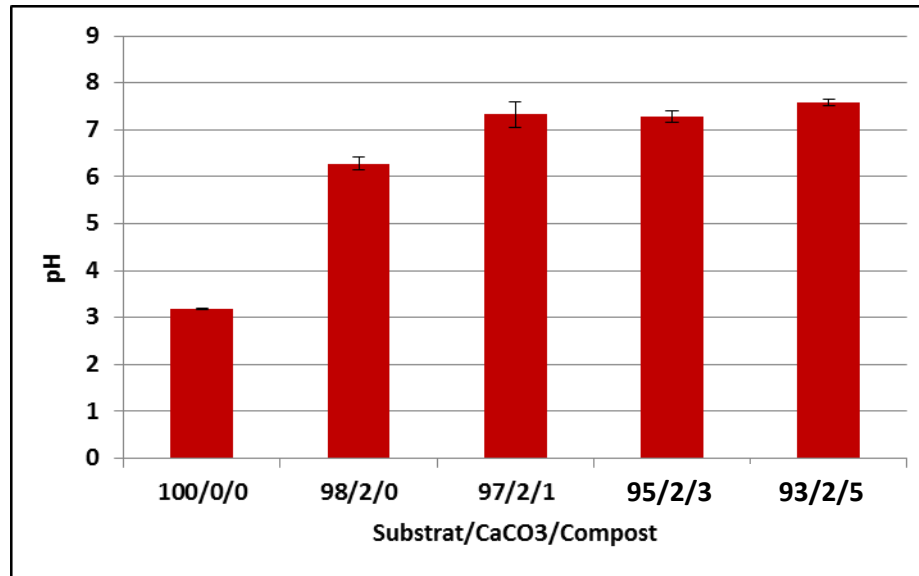
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## Complex mixture : pH effect



\* Synergistic effect of calcium carbonate /compost amendment (CaCO<sub>3</sub> alone, pH = 6.3; Compost alone 1%, pH = 5.5)

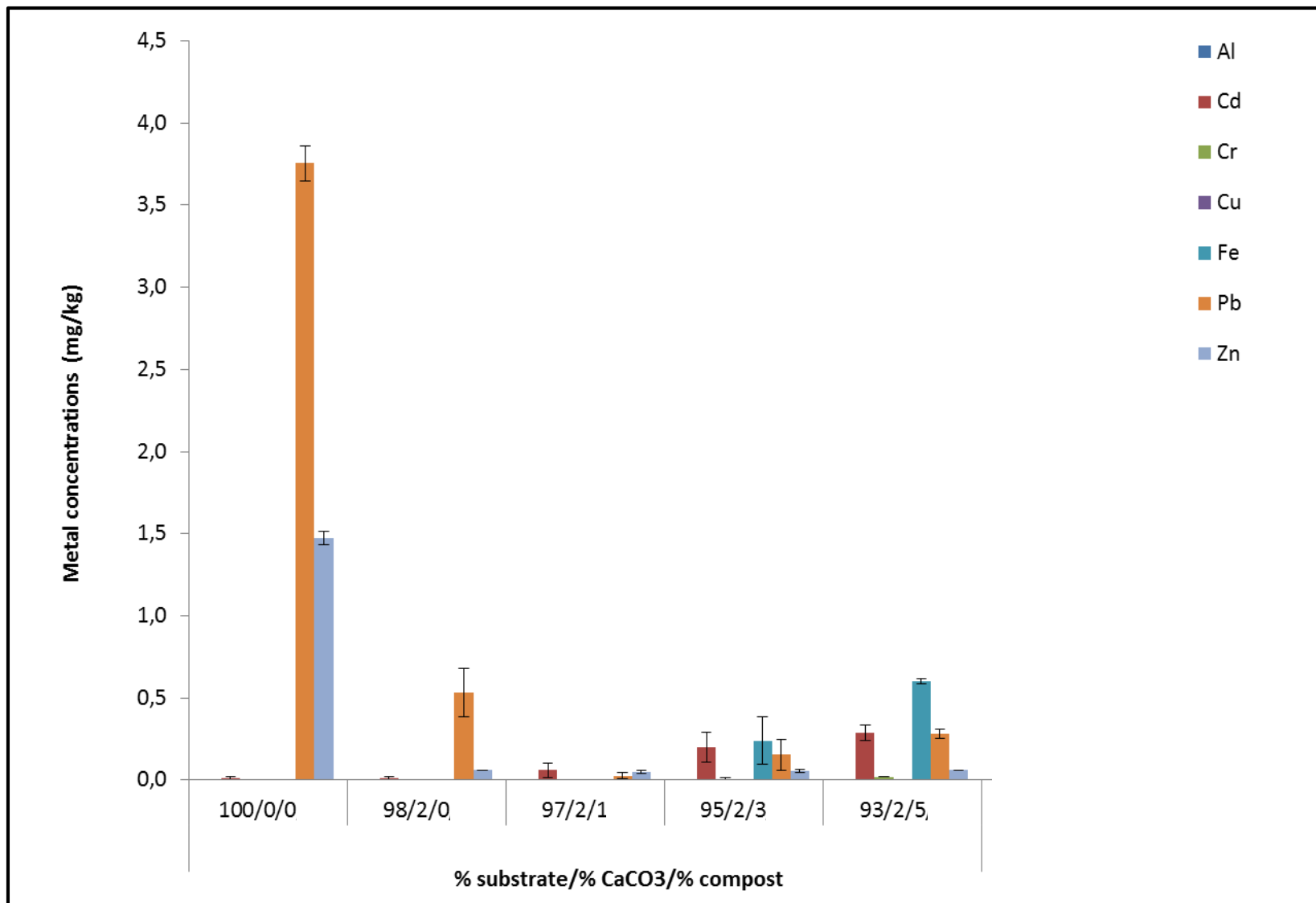
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# Complex mixture : Metal concentrations



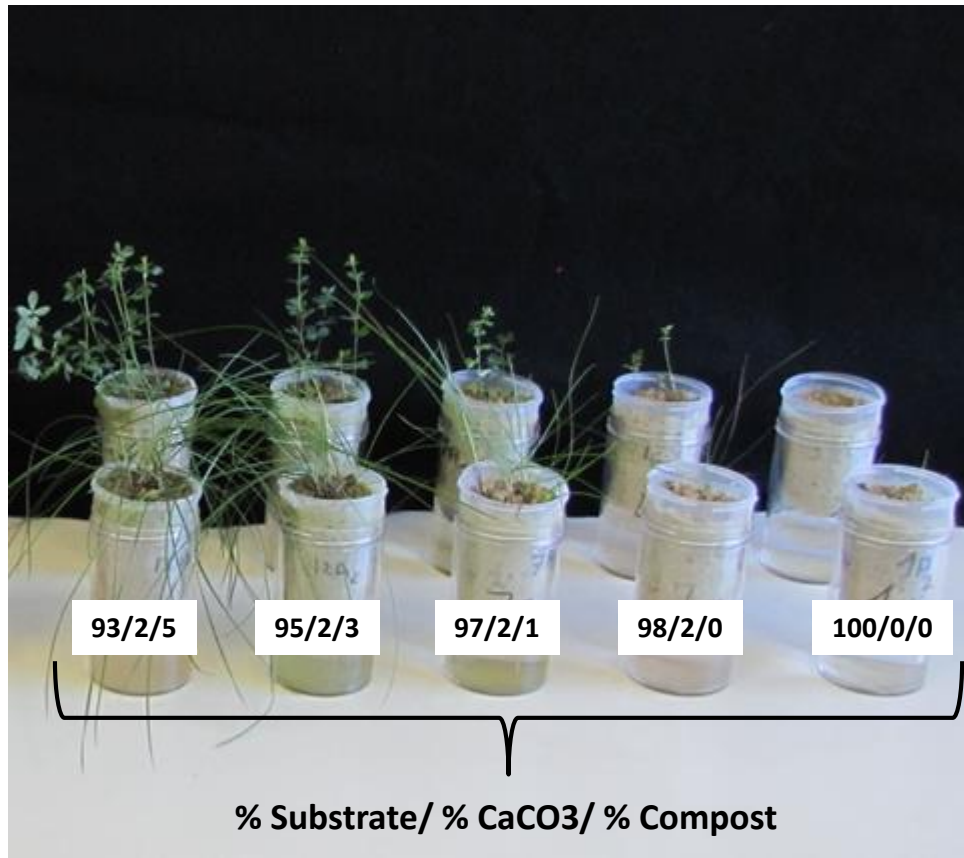
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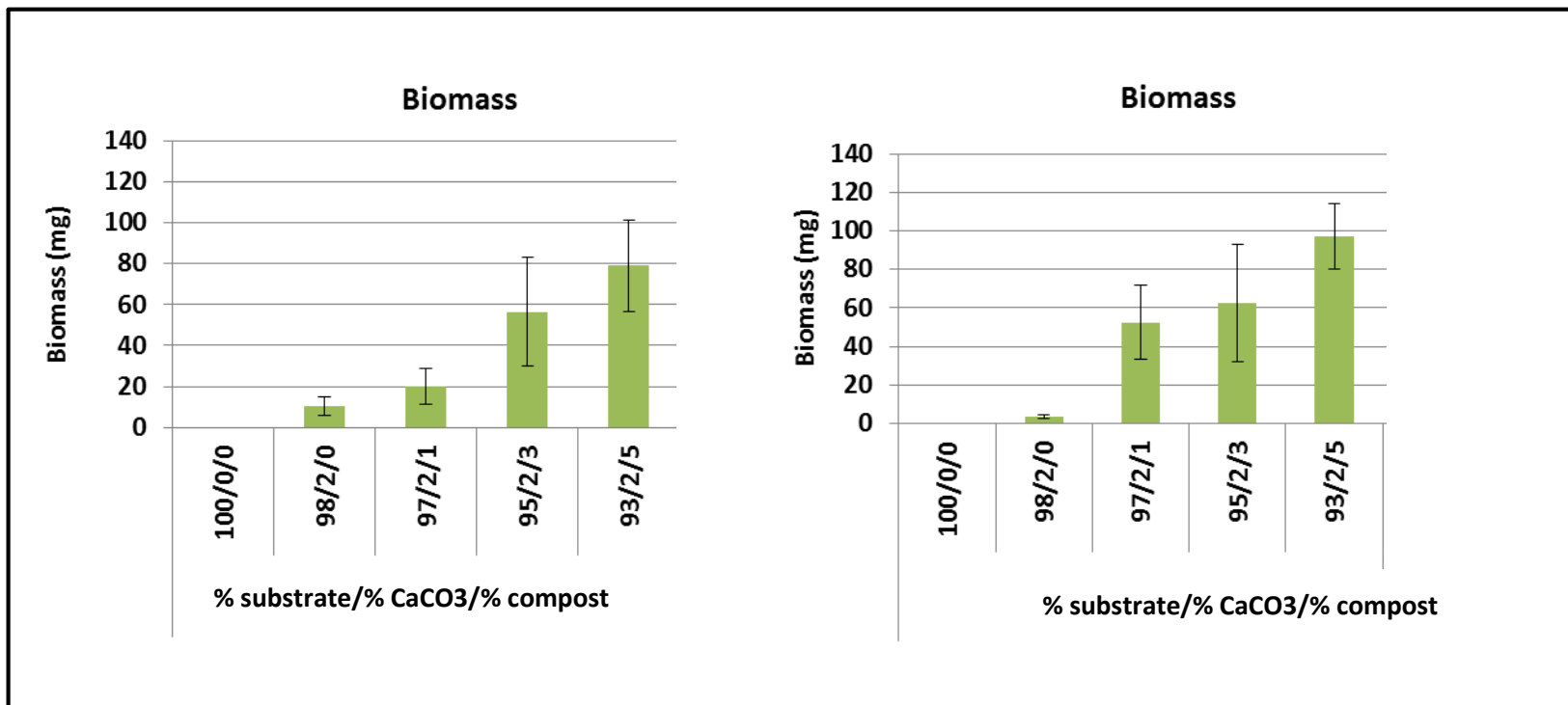
## Growth test of two plant species ( Lotus and festuca) in mini-lysimeters containing different mixtures



- 100% of mortality was observed on Lotus and Festuca grown on the Saint Sébastien d'Aigrefeuille substrate
- The addition of compost and limestone promotes growth of both species on the mine substrate

## Lotus

## Festuca



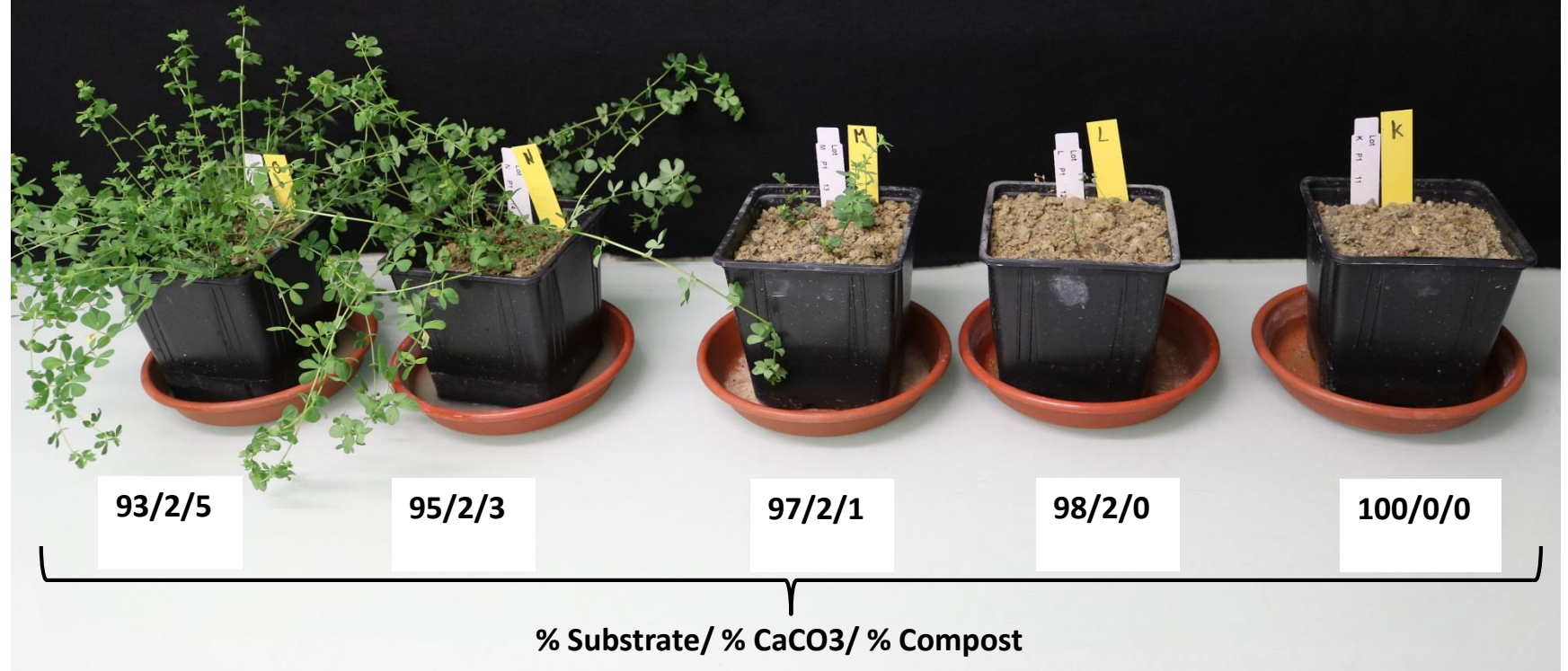
- The best dry biomass was obtained with plants cultivated on 2 % of limestone and 3-5 % of compost



- The reconstituted substrates are compatible with the nodulation of Fabaceae (*Lotus*) => mobilization of atmospheric nitrogen



# Greenhouse experiments



- 100% of mortality was observed on Lotus plant grown on the Saint Sébastien d'Aigrefeuille substrate
- The best growth was obtained with plants cultivated on 2 % CaCO<sub>3</sub>
- Compost optimizes plant growth
- The same results were obtained with Festuca

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## *In situ* experimentation



**Burying and homogenization of mineral substrate amendments**



**Experimental device after seeding plots**

**In progress...**

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# Conclusions

- ✓ Scientific knowledge and experimental design have been used to develop a practical and low cost land reclamation solution
- ✓ Necessary to monitor the pH over time in order to establish the stability of the soil conditions and sustainability of the plant growth cover.

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# Perspectives

- Analysis of metals in plant tissues
- Monitoring of experimental plots *in situ*
- Phytostabilisation of mining sites: choice of technosols; the most suitable plant species.

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# Thank you for your attention

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