Pilot scale assay for arbuscular mycorrhizal fungi-assisted phytomining

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Introduction

The increasing demand for **critical raw materials (CMR)** has intensified the search for sustainable extraction methods. **Phytomining**, an emerging green technology, uses hyperaccumulator plants to recover metals from mining soils, tailings, or contaminated sites, offering an environmentally friendly alternative to conventional mining.

Recently, **microbial-assisted phytomining** has been proposed, where microbial inoculants enhance the process by improving nutrient uptake and increasing plant tolerance to heavy metals (HMs). Among them, **arbuscular mycorrhizal fungi (AMF)** play a key role by promoting plant growth and mitigating HM toxicity.

Our study focuses on the **Pb-Zn Montevecchio-Levante mining site**, located in the municipality of Guspini, western Sardinia, Italy. With 143 years of mining activity over 25 ha, and its location in a native forest area, **this site has the potential for assays** with this novel environmental **sustainable extraction technique** for metals and CMR.

Objective

Test a sunflower–AMF system using a novel inoculation methodology to optimize conditions for effective phytoextraction in Montevecchio soil.

Methodology

8 sunflowers

Treatments

Mvc + AMF	Commercial + AMF
Mvc	Commercial

AMF inoculum made from native spores of the mine.

Bioreactors were filled with a mixture of 5 soil samples of Montevecchio soils.

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Physicochemical proprieties and chemical elements content in Montevecchio soil

3 months growth

Harvest biomass

Fresh and dry weight of roots and aerial part

Dried in 105°C until constant weight

Frecuency and intensity of AMF colonisation

Stain of roots with Trypan Blue according to the modified protocol Phililps and Hayman (1970).

Metal content of roots and aerial part

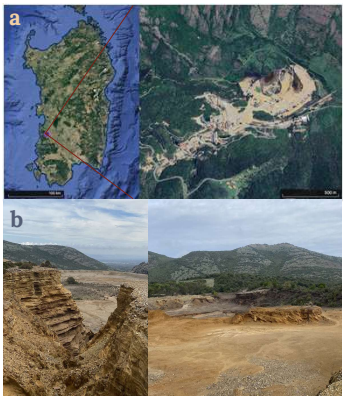
By acid digestion and inductively coupled plasma – atomic emission spectrometry

Results

Montevecchio physicochemical soil properties

Soil texture	silty loam
pH	6,2 ± 0,1
EC (uS/cm)	1082,67 ± 26,08
Salinity (psu)	0,54 ± 0,01
TDS (ppt)	530,8 ± 11,93
%H	0,84 ± 0,01
%MO	3,41 ± 0,17
TC	96,59 ± 0,17

Mean ± sd, n = 3.

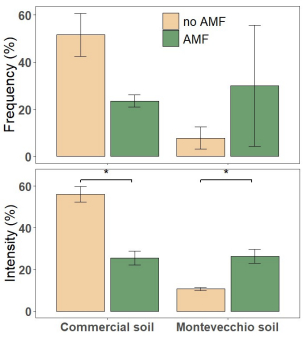


a. Geographical location of Montevecchio in Sardinia, Italy (left, bar = 100km) and Montevecchio mining site (right, bar = 500m). **b.** Views from inside the mine.

Chemical elements (*mg/kg*)

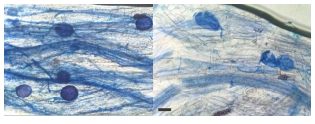
As	101,5 ± 14,8
Cd	52,1 ± 34,0
Co	47,8 ± 10,0
Cu	143,4 ± 58,2
Hg	1,06 ± 0,43
Pb	1361,2 ± 607,7
Sb	28,7 ± 14,2
Zn	6609,4 ± 3133,1

Mean ± sd, n = 5.

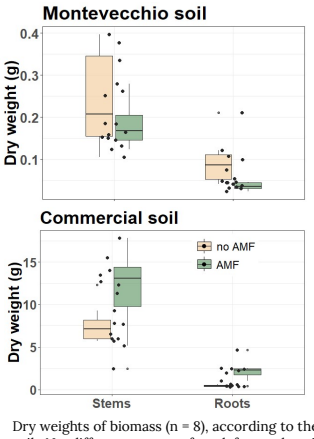


Percentages of frequency (up) and intensity (down) of colonisation by AM fungi on sunflowers in commercial and Montevecchio soil. Means ± sd (indicated by bars, n = 4). ANOVA of 2 factors was carried and comparisons inside soils showed significant differences for intensity levels in both.

Bioreactors filled with Montevecchio or commercial soil, and sowed with sunflower with or without inoculation. Treatments from left to right: commercial + amf (C+amf); Montevecchio + amf (Mvc+amf); commercial (C); and Montevecchio (Mvc).



Arbuscular mycorrhizal (AM) colonisation in roots of plants from Mvc+amf treatment. Intraradical spores and hyphae are shown. Bar = 40 µm.



Dry weights of biomass (n = 8), according to the soil. No differences were found for each soil and plant parts between inoculation treatment, but there were differences between soils in both biomass parts.

The Montevecchio soils were slightly acids (pH = 6.2) and low in organic matter content. The concentrations of metals in the soil samples used in the bioreactors exceeded the reference limit values established by Italian legislation. Metal concentrations on biomass are still ongoing.

The stem and root dry biomass of sunflowers grown in mining substrate did not show significant differences among the inoculation treatment. However, there was a trend toward increased AMF colonization frequency and a significant increase in colonization intensity for inoculum application treatment in Montevecchio soil.

This is the first trial on Montevecchio soils for phytoextraction, with a perspective on phytomining due to the potential CMR content in these soils. Further studies using new amendments in the substrate and/or other plant species are needed to refine and optimize the conditions for this environmentally sustainable method.

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