Effects of the long-term phytomanagement of Cu mine-soils on microbial diversity and soil quality

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PhytoSUDOE

Demonstrating improvements in soil biodiversity, functionality and ecosystem services of contaminated and degraded land under (phyto)management within the Interreg Sudoe region.

Project objectives:

• Establish a network of contaminated/degraded sites under phytomanagement within the Interreg Sudoe region
  - Maintaining medium- to long-term field sites
  - Implementing new sites
• Characterise and demonstrate enhancements in biodiversity, soil functionality and ecosystem services through the phytomanagement
PhytoSUDOE network of field sites

Industrial zones (wood treatment facility)
- Rhizoremediation
- Phytostabilization
- Phytoextraction

Urban / Peri-urban areas
- Phytostabilization
- Sustainable re-vegetation

Mining areas
- Phytostabilisation
- Phytoextraction
Touro Cu mine – (Galicia, NW Spain)

- Active from 1974 – 1988
- Now confined to extraction of material for road construction
- Mine tailings 550 ha
- Geological substrate is amphibolite, metal sulphides (pyrite, pyrrhotite, and chalcopyrite).
- Spolic Technosols (Episkeletic)
- Climate Atlantic (oceanic)
- Mean annual precipitation 1900 mm
- Mean annual temperature of 12.6°C.
Aided Phytostabilisation

Metal(loid)-excluding plants for the in-situ stabilisation of metal(loid)s in soils in combination with soil amendments.

- Established end of 2010
- FP7 Greenland project (Gentle remediation of trace element contaminated land)
- Tratamientos Ecológicos del Noroeste s.l. (TEN s.l.)
- SME company (established 2004)
- Recycling and valorisation of non-toxic waste products
- Organic amendments (based on organic residues and waste products)
RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SO

- Phytomanagement using short rotation coppice system (for biomass production) and different organic amendments

Soil quality

- Plant nutrients
- Organic matter
- Physical properties
- Reduce Cu phytotoxicity
- Microbial diversity

Ecosystem services

- Carbon sequestration
- Nutrient cycling
- Habitat and gene pool
- Water storage and purification
- Biomass production
Mine tailings soil properties before implementing phytostabilisation trials (Time=0)

<table>
<thead>
<tr>
<th>Untreated soil</th>
<th>Mean</th>
<th>Range</th>
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<tbody>
<tr>
<td>pH</td>
<td>3.1</td>
<td>2.7 – 3.6</td>
</tr>
<tr>
<td>%C</td>
<td>0.60</td>
<td>0.10 – 1.08</td>
</tr>
<tr>
<td>%N</td>
<td>0.10</td>
<td>0.08 – 0.13</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Texture</th>
<th>Sandy loam</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC (cmol(+)/kg)</td>
<td>21.4</td>
</tr>
<tr>
<td>Exc. Al (cmol(+)/kg)</td>
<td>8.0</td>
</tr>
<tr>
<td>Exc. H (cmol(+)/kg)</td>
<td>9.4</td>
</tr>
<tr>
<td>P olsen (mg/kg)</td>
<td>2.5</td>
</tr>
<tr>
<td>Cu</td>
<td>598.0</td>
</tr>
<tr>
<td>Mn</td>
<td>823.0</td>
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<tr>
<td>Ni</td>
<td>43.0</td>
</tr>
<tr>
<td>Cr</td>
<td>96.0</td>
</tr>
</tbody>
</table>

• Sulphide oxidation: hyperacidic, hyperoxidising soils/waters
• High [metal sulphates], PTE.
Field plot design

3 experimental blocks - 500 m²

- PC: amended with compost
- PT1: waste mixture 1
- PT2: waste mixture 2

- Sub-plots 5 x 5m
- Spacing 1 m between sub-plots; 4 m between blocks
Soil amendments

PC: composted sewage sludges, pine bark chips

Waste mixtures (PT1 and PT2):

- Sewage sludge (Vigo)
- Sewage sludge (Vilagarcia)
- Paper sludge
- Sewage sludge (CaO stabilised)
- Biomass ashes (paper mill)
- Sand (quarry residues)

Less durable effect?

### PT1
- Fe oxyhydroxides (ferrihydrate type)

### PT2
- Al slag

- Stabilise organic material (organo-mineral complexes)
- Different reactive surfaces for Cu immobilisation
Plant species

Salix viminalis

Agrostis capillaris cv. Highland

3 replicate sub-plots per species and per amendment
Progress

Incorporation amendment

Plantation

Salix - 2017

Agrostis - 2017

2011 2012
Plant-microbial-soil system

Soil physico-chemical properties

- General properties: pH, texture, CEC, C, N, pseudo-total [metal], organo- and Fe/Al (hydro)oxides
- Metal availability: H$_2$O-, NH$_4$NO$_3$-extractable [metal], BCR fractionation
- Nutrient availability: Available P (olsens), water-extract: DOC, TN, anions (NO$_3^-$, NH$_4^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$)

Soil biological properties

- Structural diversity (Bacteria, Archaea, Fungi)
- Functional diversity (enzyme activities, respiration, Biolog Ecoplate); NGS shotgun

Plant growth

- Biomass production
- Nutrition, metal accumulation
Plant-microbial-soil system

Soil physico-chemical properties

General properties

Metal availability

Nutrient availability

Soil physico-chemical properties

Structural diversity (Bacteria, Archaea, Fungi)

Functional diversity (enzyme activities, respiration, Biolog Ecoplate); NGS shotgun

Plant-microbial-soil system

Monitoring T=0, 1 yr, 2 yr, 3 yr, 6 yr

PHYTOMANAGED MINE-SOIL
- Different amendments (PC, PT1, PT2)
- Different plant covers (Salix, Agrostis)

1-3 years general amendment effect no plant species effect

Soil biological properties (microbial diversity and activity)

- Untreated soils (UNT)

NON-CONTAMINATED SOIL
- Reference soil (Oak forest, similar geological substrate (amphibolite))
Improvements in soil fertility

**RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE**

- PT1/PT2 > PC
- Durable pH effect
- PC UNP < pH
- 6 yrs: >pH under plant cover
Improvements in soil fertility

CEC (cmol_c kg^{-1})

1 YEAR

3 YEARS

6 YEARS

PC

PT1

PT2

Reference

Untreated

Unplanted

Salix

Agrostis

Unplanted

Salix

Agrostis

Unplanted

Salix

Agrostis

Unplanted

Salix

Agrostis

Unplanted

Salix

Agrostis

H

Al

Ca

K

Mg

El proyecto PhytoSUDOE (SOE1/P5/E0189) está financiado por el Fondo Europeo de Desarrollo Regional (Comisión Europea) a través del V programa Interreg Sudoe.
Improvements in soil fertility

Total C (%)

- Same trends observed in total N
Improvements in soil fertility

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

P Olsen (mg kg⁻¹)
Improvements in soil fertility

Water-extractable K (mg L⁻¹)

Water-extractable NO₃⁻ (mg L⁻¹)
Reduction in Cu availability

Immobilization

NH₄NO₃-extractable Cu

Cu-Fe/Al amor. oxides
Organo-Cu
Cu labile

Co bl
PT1/2-amended blocks: Cu associated with amorphous Fe/Al oxides
C stabilization processes

- DOC > under plant cover
- Reactive Fe phases present in all amended soils (in line with geology)
- Important phase of organo Al complexes in PT2
Improvements in soil microbial activity

- Community level physiological profiling (Biolog Ecoplates)
- AWCD> phytomanaged soils
- AWCD and C substrate use >under plant cover

**RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE**

- C substrate usage compared to UNT

![Graph showing comparisons between different conditions](image-url)
Improvements in soil microbial activity

- Catalase / Dehydrogenase activities
- C, N, P, S cycles
- T=0, almost undetectable
- Increase after addition of compost
- Plant-induced effect
- Higher activities under Salix

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**Dehydrogenase (μmol INTF g⁻¹ h⁻¹)**

**Invertase (μmol glucose g⁻¹ h⁻¹)**
Changes in soil microbial diversity (NGS sequencing illumina)

- Assignation of sequences to phyla
- Dominant phyla: Proteobacteria, Acidobacteria, Actinobacteria
- Increase relative abundance of bacteroidetes PC/PT1
- Increase halanaerobiales
Family level

PHYТОMANAGEMENT IN SOUTHWEST EUROPE

Taxa
- Nitrosonomadaceae
- Cytophagaceae
- Planctomycetaceae
- Flavobacteriaceae
- Chitinophagaceae
- Xanthomonadales_Incertae_Sedis
- Blastocatellaceae
- Xanthomonadaceae
- Oxalobacteraceae
- Gemmatimonadaceae
- Hyphomicrobiaceae
- Comamonadaceae
- Sphingomonadaceae
- Rhodospirillaceae
- Bradyrhizobiaceae
- Xanthobacteraceae
- Haliangiaceae
- Acidobacteriaceae
- env.OPS.17
- H16
- Tepidisphaeraceae
- Nitrospiraceae
- Nocardoidaceae
- Hyphomonaclaeaceae
- Caulobacteraceae
- Opitutaceae
- Sphingobacteriaceae
- Pseudomonadaceae
- Anaerolineaceae
- Micrococaceae
- Bilrii41
- Rhizobiales_Incertae_Sedis
- Elev.16S.1332
- Chthoniobacteriaceae
- Micromonosporaceae
- Others
Non-metric multidimensional scaling (NMDS) ordination plot:

- Separation of plant species/treatments

Shannon diversity > under plant cover

$H, p = 0.028$

Unplanted (UNP)