RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Progression dans l'application de stratégies novatrices de phytomanagement aux zones contaminées de l'Espace Sudoe

GT2 report

1/04/2020 – 30/04/2023 (SOE4/P5/E1021)

Product 2 &

Entregable/Livrable E2.2

E.2.2.1. Assessment of the state of the network of phytomanaged sites

Deliverable Version:	L./E.2.2.1 , V.01
Dissemination Level:	CO
Author(s):	Michel Mench, Gaelle Capdeville, Séverin Jouveau, Régis Burlett, Sylvain Delzon, Yerslan Token, Emma Clément, Sophie Beaujean, Mathilde Henrion, Raphael Segura, Florian Delerue, Beatriz Rodriguez, Angeles Prieto Fernandez, Carmela Monterroso, Lur Epelde, Carlos Garbisu, Souhir Soussou, Juan Vilela, Jose Maria Becerril, Manu Soto, E. Urionabarrenetxea, N. Garcia-Velasco, U Izagirre, B. Zaldibar, Antonio Hernández, U. Artetxe, M. Anza, Oihana Etxeberria, N Garcia, I Marigómez, Helena Gomes-Moreira, Paula Castro, Laurent Dudoit, Barbara Faure, Alvaro Nunes de Sousa, Tiago Luz, Rita Tentugal

Project Acronym	Phy2SUDOE
Project Title	RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE (SOE4/P5/E1021)
Project Coordinator	Dr. Carlos Garbisu Neiker, Department of Conservation of Natural Resources, E-48160 Derio, SPAIN, cgarbisu@neiker.eus
Project Duration	Start date: 01/11/2020 - End date: 30/04/2023

Deliverable No.	GT2 Product 2.1, Product 2.2, E.2.1.1 & E 2.2.1	
Diss. Level	Confidential	
Deliverable Lead	INRAE (UMR BIOGECO INRAE 1202)	
Status		Working
	X	Verified by other partners
		Final version
Due date of deliverable	30/04/2023	
Actual submission date	30/04/2023	
Work Package	GT2	
WP's Leader	Leader GT2: Dr. M Mench INRAE UMR BIOGECO INRAE 1202 Univ. Bordeaux, Bat B2, allée G. St-Hilaire, CS50023, F-33615 Pessac cedex, France. michel.mench@inrae.fr	
Contributing beneficiary(ies)	INRAE (UMR BIOGECO), CSIC, USC, Département Charente, Univ. Basque County, CEA Vitoria-Gasteiz, UCP-ESB, Fertil'Innov Environnement, Neiker Tecnalia, Clover strategy Associated partner: ville de Bordes, Département Pyrénées Atlantiques – CD64, Lyonnet SA, Bordeaux Métropole Partners: Bordeaux INP, Suez Environnement Lyre, Jardins de l'Angoumois, HPC Environtec, ADEME	

Introduction

This GT2 is extending the previous network of phytomanaged sites in the SUDOE region (PhytoSUDOE), proving the efficiency and the limits of phytotechnologies to remediate contaminated soils. The Phy2SUDOE network has integrated 8 new sites with new case studies (i.e. other soil uses, organic pollutants, and mixed contamination) with various edaphic conditions and future land use (https://www.phytosudoe.eu/en/the-project/sites/). These are mining areas and urban and industrial areas to broaden the range of future land uses (i.e. peri-urban green belts, parks, industrial crop production, remediated grassland, etc.). These sites depend on partner and/or associated administrations and companies, facilitating the transfer of results. New phytomanagement solutions were applied at these sites according to the PhytoSUDOE and Phy2SUDOE methodologies and gained knowledge, notably in compliance with the Phy2SUDOE GT1 for the site characterization and tools and with the GT3 as the components of the biodiversity were characterized and preserved. The objectives were:

Objectives:

(1) Evaluate pollution links: sources, exposure routes and risks (INRAE, Charente, CSIC, USC, UCP, UPV, and NEIKER).

(2) Feasibility of solutions based on phytotechnologies (NEIKER, USC, CSIC, UPV, CEA, INRAE, Fertil, Charente, UCP, and Clover strategy)

(3) Remediation / Phytomanagement strategies: operation plan, evidence of effectiveness, benefits / limitations of crops, soil functions and services (NEIKER, USC, CSIC, UPV, CEA, INRAE, Fertil, Charente, UCP, Clover)

Activity 2.1/ Deliverable E.2.1.1 Characterization of new sites integrated to the "Network of phytomanaged sites in the SUDOE region", to add new case studies and phytomanagement strategies

These works and results reflect the conformation and status of eight new sites extending the network of phytomanaged sites in the SUDOE region, with new case studies, (associated) partners, stakeholders, and future land uses.

As a reminder, in total the network was extended to 15 sites (7 PhytoSUDOE + 8 Phy2SUDOE). The human capital of the network was expanded with partners of different types (universities, R&D centers, companies, and administrations) to stimulate the transfer of results. Each site will have its own action plan: conceptual model, strategies, protocols, monitoring, etc.

Regarding site history, soil contaminant and sources, vegetation present, conceptual model and future land use, more details are given for each site in the deliverable E.1.1.1 – Status of the Network.

Activity 2.1 focused on characterizing the 8 new sites added to the network. This characterization included the following aspects (participants in parentheses):
(1) the collection of available site information (CSIC, USC, UPV, CEA, INRAE, Charente, Fertil, UCP) ;

(see also Deliverable E.1.1.1. for each site)

(2) physico-chemical and biological soil properties (CSIC, USC, UPV, CEA, INRAE, Charente, UCP, NEIKER)

(3) to identify and quantify the contamination sources (total and bioavailable concentrations of contaminants), exposure routes and potential risks for biological receptors (CSIC, USC, UPV, CEA, INRAE / Charente, UCP, NEIKER); (see also Deliverable E.1.1.1. for each site)

(4) development of a conceptual site model (CSIC, USC, UPV, CEA, INRAE, Charente, Fertil, UCP)

(see also Deliverable E.1.1.1. for each site)

(5) discussion with site managers/owners on the particular uses and interests of each site (all).

The new sites are (responsible for each site in brackets):
- ES: Bandeira – (ultramafic) quarry: Ni, Cr (CSIC, USC)
- ES: Gernika - mixed contamination due to uncontrolled spreading of sewage sludge (UPV)
- ES: Zumabakotxa - peri-urban and industrial area with mixed contamination (CEA)
- FR: Sentein-Bulard - mining area: Pb, Zn (INRAE, in collaboration with Bordeaux INP)
- FR: Durandeau - industrial and neighboring area (edge of the Charente estuary): mixed pollution (Charente)
- FR: Les Avinières - mining area: Pb, Zn, Cd, Ni (Fertil)
- FR: Bordes – former landfill, mixed pollution (INRAE, with CD64, Bordes town and Suez lyre)
- PT: Estarreja - industrial area: mixed pollution (UCP)

● Conceptual models and operations planned: all information were produced in the previous reports delivered in March and June 2022 and in the deliverable E.1.1.1. (Status of the Network) for all site

Activity 2.2/ Deliverable E.2.2.1 Assessment of the state of the network of phytomanaged sites
In collaboration with WG1, this integrative document was developed. It showed the status of the network in terms of pollution risk reduction, product generation and service provision. The objective was to provide field data on the usefulness of plant management to remediate degraded sites and to promote the use of the ph2SUDOE network as a "demonstration pilot" for all those interested in phytomanagement. Following points were addressed:

(1) Selection and definition of phytomanagement solutions. Among others, the following options were evaluated: phytostabilization with metal-excluding plants; phytoextraction with metal-accumulating plants; phytomining with high-value metal-accumulating plants; rhizo/ biodegradation through the stimulating effect of root systems on soil microbial communities; bioaugmentation with microbial consortia with the ability to promote plant growth; and biostimulation with organic and/or mineral amendments (all) ;

(2) Implementation of phytomanagement solutions (CSIC, UPV, CEA, INRAE, Charente, Fertil, UCP)

(3) Physicochemical, ecotoxicological and biological characterization of soils (CSIC, USC, UPV, CEA, INRAE, UCP, NEIKER)

(4) Quantification of total and bioavailable concentrations of pollutants (CSIC, USC, UPV, CEA, INRAE, UCP)

(5) Estimation of potential products generated (CSIC, UPV, CEA, INRAE, Charente, Fertil, UCP)

(6) Estimation of ecosystem services generated (CSIC, USC, UPV, CEA, INRAE, Charente, Fertil, UCP)

	Contaminants	Soil	Plants	Earthworms Animals tests	Soil DNA/ microbes	Remediation Actions	Risk assessment (RA)
NS 1 – Durandeau (FR)	TCE, Ni, Cu, Pb, Cd, Zn, PCB, PAH	√	Miscanthus, vetiver, aster, poplar, willows, alfalfa, ryegrass, carex, Agrostis	Toxicity: nematode Nematofauna	Bacteria, fungi	Phytomanagement ongoing (phytostabilization, biodegradation) Compost, bioaugmentation Lixiviates collected with lysimeters	RA done
NS2 - Les Avinières (FR)	Zn, Pb, Cd, As, Tl	√	Metallophytes pseudometallophytes		Mesorhizobium	Phytomanagement (phytostabilization, bioaugmentation) ongoing compost	RA done
NS3 – Sentein (FR)	Zn, Pb, Cd, (As)	√	Metallophytes pseudometallophytes		Done Neiker Bacteria Biolog and soil enzymes (CSIC)	Plant survey Feasible phytostabilization options assessed in pot trials Compost, biochar, dolomite, bioaugmentation (bacteria, earthworms)	RA done
NS4 – Bordes (FR)	Metal(loid)s, PCB, PAH	√	White clover, ryegrass, local trees, bioaugmentation of seed bank by hay & soil transfer	Toxicity assessed on earthworms Nematofauna	Soil DNA Nematofauna Bacteria (Neiker)	Phytomanagement ongoing: phytostabilization, biodegradation, bioaugmentation of	RA, local grassy & woody excluders

				Daphnia on leachates	Biolog and soil enzymes (CSIC)	seed bank by hay & soil transfer Compost, biochar, dolomite	
NS5 – Bandeira (ES)	Ni, Cr	√	Ni hyperaccumulators			Phytomining (bio)monitoring Compost	RA done
NS6 – Guernika (ES)	Cd, Cr, Ni, Pb, PAH, dieldrin	√	3 plant species (alfalfa	Toxicity reproduction biomass, root elongation	Biology and soil enzymes (CSIC)	Phytomanagement ongoing : phytostabilization, biodegradation, bioaugmentation	RA done Feasible options
NS7 – Zumabakotxa (ES)	As, Pb, PCBs PAH, acetone, hydrocarbons	√	alfalfa meadow, Gall oak forest, Holm oak forest, willow/poplar stand, scrubland		Soil Card	Phytomanagement ongoing : phytostabilization, biodegradation compost	RA, done
NS8 Estarreja (PT)	aniline & derivatives, BTEX, PAH, ammonia, As, Hg, Pb, Zn	√	Mycorrhizal Willows, poplars	mesofauna	Bait lamina	Phytomanagement ongoing : phytostabilization, biodegradation compost, hydrogel	RA done

● Methodologies for characterizing physico-chemical and biological activities are listed in the following table and is harmonized with those in the GT1.

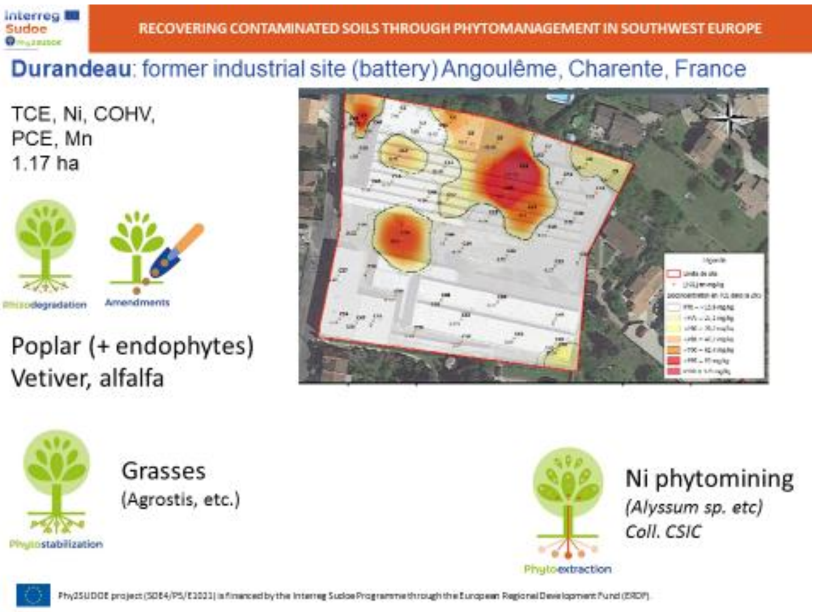
			NS1	NS2	NS3	NS4	NS5	NS6	NS7	NS8
Physic-chemical properties	**Soil pH** (1:2.5 soil:wáter; or 1M KCl)		X	X	X	X	X			
	CEC	Cobaltihexamine method	X	X	X	X				
		1M NH_4Cl (Ca, Mg, Na, K), 1M KCl (H, Al), AAS/ICP-OES					X			
	Extractable P (Olsen's $NaHCO_3$method)		X (Olsen)	X (joret - Hébert	X (Olsen)	X (Olsen)	X			
	Phosphorus speciation/fractionation									
	Total C and N (Combustion, LECO analyzer)		X	X	X	X	X			
	SOM fractionation									
	Carbonates (Gravimetry, Schleiber method)		X	x	X	X	NA			
	Fe/Al oxi(hidroxi)des (Selective extraction methods for Fe/Al oxi(hidroxi)des)									
	Bulk density									
	Soil moisture		X							
	Water-holding capacity		X							
Trace elements and organic contaminant	Bioavalable TE (EDTA, H2O,..)	NH_4Cl					x			
		NH_4NO_3	x	x	x	x				
		H_2O								
		EDTA								
		DTPA					X			
		$Sr(NO_3)_2$					X			
	Total TE (H_2O_2/3:1 HCl:HNO_3, microwave)		X (HF)	X (Microwave)	X (HF)	X (HF)	X		X (VIE-B)	
	Soil metal fractionation (modified BCR protocol)									
	Total PAH (Hexane extraction, GC-MS determination)		x			X	NA		X (VIE-B)	
	other organic contaminants		PCB, VOC (TCE), BTEX			PCB, BTEX	NA		X (VIE-B)	
Biological and biochemical properties	**Enzymatic activities**		(done by CSIC)		(done by CSIC)	(done by CSIC)				
	CLPP (Ecoplates Biolog ™)		(done by CSIC)	(done by CSIC)	(done by CSIC)	(done by CSIC)	X	X		
	Respiration							X		
	microbial communities	extraction Soil DNA		extraction Soil DNA microbial communities						
	in situ /ex situ test	e.g. Bait-Lamina and other ones								
	Ecotoxicity test (germination, microtox...)		germination	germination	germination	germination				
	Potentially mineralizable nitrogen							X		
	Soil fauna		invertebrates, nematodes		invertebrates	invertebrates				
Plant analysis	**Biometric parameters**		X*	X	X*	X*	X		X**	
	TE concentration		X	X	X	X	X			
	Physiological and pigment parameters		chlorophyll index	LEAF FLUORESCENCE (SPAD)					X***	
	Nutrients		P, K, Ca, Mg, Na	P, K, Ca, Mg, Mn, Na	P, K, Ca, Mg, Na	P, K, Ca, Mg, Na	X			
	TEs bioconcentration and translocation factors		X		X	X				
	Soil health cards								X	

*	leaf area index	**	total photosyntethic area	***	photosyntethic efficiency
	maximum shoot length		leaf area index		chlorohyll
	DW yield of plant parts		maximum shoot length		carotenoids
			DW yield of plant parts		tocopherols

Table. Tools /methodologies for assessing physico-chemical and biological activities

NS1 Durandeau site:

● **Implementation**: organic and inorganic contaminants were firstly assessed by La Charente (and HPC Envirotec) in soil and subsoil on the whole site and mapped. Then the concrete slab was removed in March 2022 on roughly 200 m² under the supervision of La Charente and HPC-Envirotec. Thereafter the 0 – 0.50 m soil layer was loosened. This area displayed high metal(loid), PAH, PCB, and trichloroethylene concentrations in the topsoil. Based on previous pot experiments carried out by INRAE, compost (5% w/w) was incorporated into the topsoil.



Set up of the Durandeau site in March 2022 (© Dudoit La Charente /Mench INRAE)

● **Soil properties**: this alkaline technosol displays a soil contamination by metal(loid)s (notably Zn, Ni, Cu and Cd in excess) and organic compounds, listed in the following tables:

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Durandeau (NS1, France)

Feb – May 2022

- Preparation of plants for transplantation
- Pot trial: testing of composts
- Soil analysis, plot selection
- Field trial +outdoor lysimeters: implementation

- Sandy texture (86%, clays : 4.5-7.6%), C/N: 20 - 78
- pH : 8.65 – 9.6
- Organic C: 1.2 – 5.5 %
- Zn: 299 - 5874 mg/kg
- Ni: 125 - 3850, Cu: 59 - 706, Cd: 9.6 - 111
- Hg: 8.5 - 35, As: 5 - 20, Pb: 74 - 625
- ∑PAHs: 1 – 6.4 mg/kg; ∑PCBs: 3.6 – 212 mg/kg
- Aliphatic hydrocarbons: 829 – 9630 mg/kg
- Trichloroethylene: 1.6 – 5.3 mg/kg

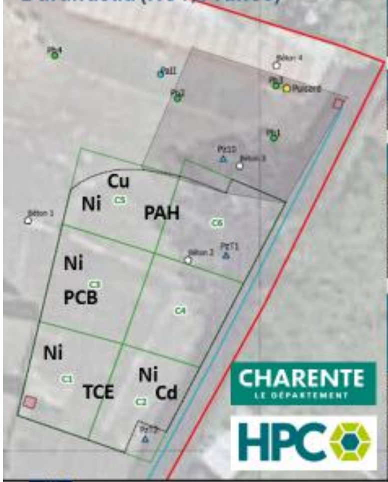
Ni-hyperaccumulators

Phytovolatilization, Phytostabilization, Rhizodegradation, Bioaugmentation

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Durandeau (NS1, France)



Field trial : soil analysis

Echantillons		C1	C2	C3	C4	C5	C6	C7
Profondeur (m)		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
Matières sèches	%	89,7	90,0	89,9	90,7	87,9	89,7	91,2
Carbone Organique Total	mg/kg MS	8 740	34 400	24 000	17 700	39 800	40 100	20 000

Hydrocarbures C_5-C_{40}		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
HC C_5-C_{10}	mg/kg MS	<1,00	<1,00	2	2	7,7	6,3	1,2
HC C_{10}-C_{40}	mg/kg MS	120	183	408	611	4 570	4 240	822

Hydrocarbures aromatiques monocyclique (BTEX)		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
Benzène	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Toluène		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Ethylbenzène		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
o-Xylène		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
m,p-Xylène		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Xylènes totaux		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Somme des 4 BTEX		<0,0500	<0,0500	<0,0500	<0,0500	<0,0500	<0,0500	<0,0500

Eléments Traces Métalliques (ETM)		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
Arsenic (As)	mg/kg MS	13,4	13	13	9,82	16,6	11,3	11,4
Cadmium (Cd)		54,6	93,1	52,4	60,1	68,8	25,3	41,4
Chrome (Cr)		17,6	17	17,9	28,1	19	16,7	18,9
Cuivre (Cu)		63	58,7	49,9	58,3	129	112	54,3
Mercure (Hg)		1,11	3,05	2,06	1,23	3,5	2,54	1,0
Nickel (Ni)		1 780	1 900	2 440	973	4 070	<100	2 580
Plomb (Pb)		135	100	108	84,9	194	159	90,9
Zinc (Zn)		124	361	159	177	324	308	145

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Durandeau (NS1, France)

Field trial : soil analysis (continued)

PolyChloroBiphényles (PCB)		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
PCB 28	mg/kg MS	<0,01	<0,01	0,56	<0,01	0,13	0,28	0,03
PCB 52		0,01	0,01	2,05	0,02	0,89	0,89	0,2
PCB 101		0,05	0,05	6,45	0,07	2,16	3,91	0,55
PCB 118		0,05	0,03	3,04	0,06	1,29	1,82	0,03
PCB 138		0,2	0,13	33,3	0,16	4,14	8,03	1,12
PCB 153		0,19	0,14	22,2	0,14	4,91	8,4	1,41
PCB 180		0,12	0,07	14	0,06	2,59	3,57	0,59
Somme des 7 PCB		0,62	0,43	81,6	0,51	16,11	26,9	3,93

Hydrocarbures Aromatiques Polycycliques (HAP)		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
Naphtalène	mg/kg MS	<0,05	<0,05	0,08	<0,05	0,54	0,62	0,44
Acénaphthylène		<0,05	<0,05	<0,05	<0,05	<0,25	0,51	<0,05
Acénaphtène		<0,05	<0,05	<0,05	<0,05	0,5	2,3	0,28
Fluorène		<0,05	<0,05	<0,05	0,06	0,28	1,2	0,13
Phénanthrène		<0,05	0,13	0,1	0,45	3,4	5	0,92
Anthracène		<0,05	<0,05	<0,05	0,1	1	2,5	0,45
Fluoranthène		0,09	0,18	0,27	0,67	6,5	15	2,3
Pyrène		0,11	0,17	0,25	0,47	5,4	14	2
Benzo(a)anthracène		0,10	0,09	0,16	0,31	3,3	9,6	0,96
Chrysène		0,13	0,12	0,19	0,36	3,9	9,4	0,94
Benzo(b)fluoranthène		0,31	0,26	0,39	0,46	10	20	1,7
Benzo(k)fluoranthène		0,09	0,09	0,13	0,18	3,3	6,4	0,63
Benzo(a)pyrène		0,17	0,18	0,24	0,25	6,6	15	1,2
Indéno(1,2,3-c,d)pyrène		0,2	0,18	0,25	0,29	7,2	15	1,3
Dibenzo(a,h)anthracène		<0,05	<0,05	0,06	0,07	1,6	3	0,21
Benzo(g,h,i)pérylène		0,18	0,24	0,23	0,32	6,6	14	1,1
Somme des 16 HAP		1,38	1,64	2,35	3,98	60,12	133,53	14,56

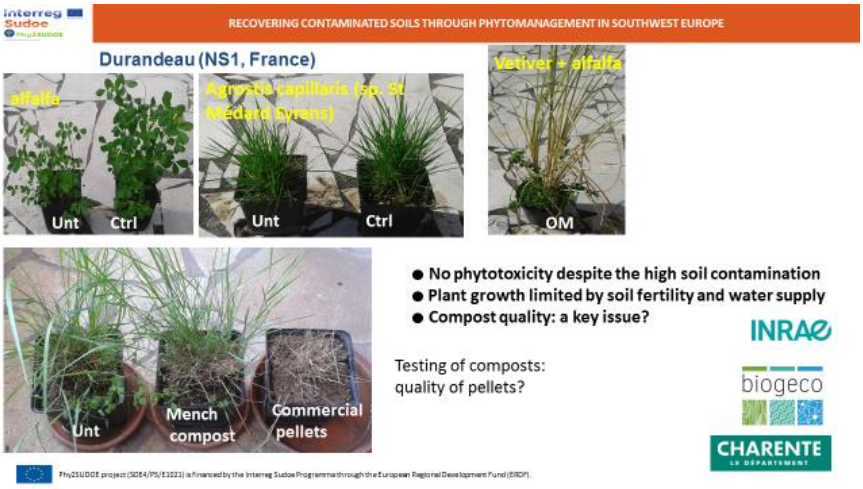
RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Composés Organo Halogénés volatils (COHV)		C1 0 - 0,5	C2 0 - 0,5	C3 0 - 0,5	C4 0 - 0,5	C5 0 - 0,5	C6 0 - 0,5	C7 0 - 0,5
cis 1,2-Dichloroéthylène	mg/kg MS	<0,10	0,28	<0,10	<0,10	<0,10	0,17	<0,10
Trans-1,2-dichloroethylène	mg/kg MS	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Trichloroéthylène	mg/kg MS	3,36	8,62	0,62	0,38	0,82	0,82	0,54
Tetrachloroéthylène	mg/kg MS	1,59	1,65	0,17	0,24	0,09	0,17	0,25
Chlorure de Vinyle	mg/kg MS	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02
1,2-dichloroéthane	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
1,1,1-trichloroéthane	mg/kg MS	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Dichlorométhane	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Tetrachlorométhane	mg/kg MS	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02
1,1-Dichloroethene	mg/kg MS	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
1,1-dichloroéthane	mg/kg MS	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
1,1,2-trichloroéthane	mg/kg MS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
1,2-Dibromoéthane	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Bromodichlorométhane	mg/kg MS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
Dibromométhane	mg/kg MS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
Trichlorométhane (chloroforme)	mg/kg MS	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02
Tribromométhane (bromoforme)	mg/kg MS	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Bromochlorométhane	mg/kg MS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
Dibromochlorométhane	mg/kg MS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
Somme des 19 COHV		4,95	10,55	0,79	0,62	0,91	1,16	0,79

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

Soil contaminants in the Durandeau site © La Charente

● **Phytomanagement options /Plant assembly:** After soil amendment, an initial plant community (i.e. mycorrhized black poplars and goat willows (Cu/PAH tolerant populations from the S1 site) , vetiver, *Miscanthus* x *giganteus*, *Amorpha fruticosa*, *Agrostis capillaris* (Cu/PAH-tolerant population of site S1), *Festuca pratensis* (Cu-and Ni tolerant population of the Louis Fargue site), *Medicago sativa*, and *Lolium perenne,* all plants prepared by INRAE was implemented to promote the phytostabilization of metal(loid)s, the Cd/Zn phytoextraction (by collecting poplar and willow leaves in autumn), rhizo/biodegradation of organic xenobiotics, and soil cover to prevent wind erosion and water runoff (March 2022). Selection of plant species was reported in previous reports and was based on pot experiments (soil phytotoxicity being very low). Soil sampling, investigation of plant community, and maintenance were realized in May, July, August and October 2022. Plant and soil samples were analyzed in Oct. /Nov. 2022. Plant traits (mortality rate, maximum shoot length, and shoot biomass) were determined in Nov. 2022. Data were presented at the 3rd workshop (Santiago de Compostela, Oc. 2022)



Plant testing on the Durandeau soils to select the initial plant community © Mench INRAE/Charente

Besides, six large lysimeters were loaned to La Charente County and implemented at this site. Three lysimeters were filled with compost-amended soil and three others with unamended soil (from the field trial) to monitor changes in the drainage water. One mycorrhized poplar and *A. capillaris* were transplanted into each lysimeter. The monitoring of this field trial was supervised by La Charente County, with the help of HPC-Envirotec and other Phy2SUDOE partners (e.g. INRAE, CSIC). In this dried spring 2022 with heatwaves, the irrigation was essential to allow the plant development.

In parallel, CSIC was testing 3 hyperaccumulators on the Durandeau soil in a pot experiments and determining the soil enzyme activity and biological soil activities (Biolog).

Oct. to Dec. 20222: Nematofauna and soil toxicity to Nematode were investigated on the untreated and phytomanaged soil after 7 months. DNA extractions were also made to determine the bacteria and fungi community Plant testing using the fading technique, rape and barley was carried out from Nov. 2022 to Feb. 2023. Plant traits and shoot ionome were investigated. Large soil sample (80kg) was sent to UCP-ESB to carry out a pot experiment with selected PGPR bacteria (bioaugmentation).

● **Success / limits**:

- Poplars, willows, and grassy species implemented in a field plot by the Charente County and INRAE partners are successfully developing and limiting the pollutant linkages. No metallophyte colonists were present. Rate of vegetation cover was close to 80% with only some bare soil remaining between the pipes of the irrigation system. Mortality rate was roughly 0% (with only one died poplar replaced, being damaged by the wind).

- Most soil contaminants (metals and organic compounds) are not bioavailable and do not accumulated in the plant shoots. Even in the leaves of poplars and willows, the Cd and Zn concentrations were unexpectedly low, likely due to the low metal availability (demonstrated by the water-leaching test) and alkaline soil pH.

- Despite the high total contaminant concentrations, the phytomanaged soil had only a slight negative effect on the growth (-9%) and reproduction (-7%) of the *Caenorhabditis elegans* nematode but not on its fertility. Based on the nematofauna, the phytomanaged soil still displays a low biological state but a good organism activity, high nutrient fluxes, a low ecological insurance (low food web complexity) and low diversity of organisms. The decomposition pathways are mostly dominated by the bacterial community. On site, no invertebrate were noticed in the soil and the vegetation cover.

- The phytomanagement induced changes in the diversity of soil bacteria and fungi communities. The bacteria and fungi communities were identified (by targeting specific regions of their DNA) and their relative abundance semi-quantified in the soil before and after the compost incorporation and implementation of the plant community. The diversity of bacteria strains was high in this untreated soil, notably as compared to other agricultural metal-contaminated soils, showing a particular biodiversity to preserve. *Actinomarinicola tropica* (2.7%), *Sphaerobacter thermophilus* (2.6%), *Vicinamibacter sylvestris* (2.1), and *Paludibaculum fermentans* (2%) had the highest relative abundance in the untreated soil. The diversity of bacteria strains was reduced in the phytomanaged soil after 7 months. *Pseudomonas flexibilis* was dominant (27%), and *Lederbergia lenta* (2.2%), and *Sphaerobacter thermophilus* (2%) in a lesser extent, in the phytomanaged soil. Four out of 14 identified fungi were dominant in the untreated soil: i.e., *Aspergillus aureolus* (12.2%), *Plagiomnium medium* (10.7%), *Sordaria equicola* (8.8%) and *Paludibaculum* *fermentans* (2%). Twenty two fungi strains were identified in the phytomanaged soil, *Scopulariopsis cordiae* (28.7%), *Echria gigantospora* (11.8%), *Tricharina praecox* (10.5%) and *Phaeoisaria filiformis* (3.8%).

Bacteria and fungi populations adapted to contaminant exposure are preserved in untreated areas. Potential contaminant leaching out of the root zone is monitored using planted lysimeters.

The field trial implemented at Durandeau site in May 2022 (© Jardins de l'Angoumois)

Lysimeters and field plot in October 2022 (© Mench INRAE/ Dudoit La Charente)

(Left) Potential symptoms of negative effect of TCE exposure on bottom poplar leaves and (Right) green leaves at the top of poplars © Mench/INRAE

NS2- Les Avinières:

The site is located in the Malines mining district in the Saint-Laurent-le-Minier region (Gard). This district is the largest lead and zinc mining basin in France. Since its closure in 1914, the Avinières mine site has not benefited from any redevelopment operations, and the toxicity of the mine's soils has long been ignored. Part of the site was thus sold in the 1990s to a farmer by a public body, SAFER, so that he could develop a market gardening activity

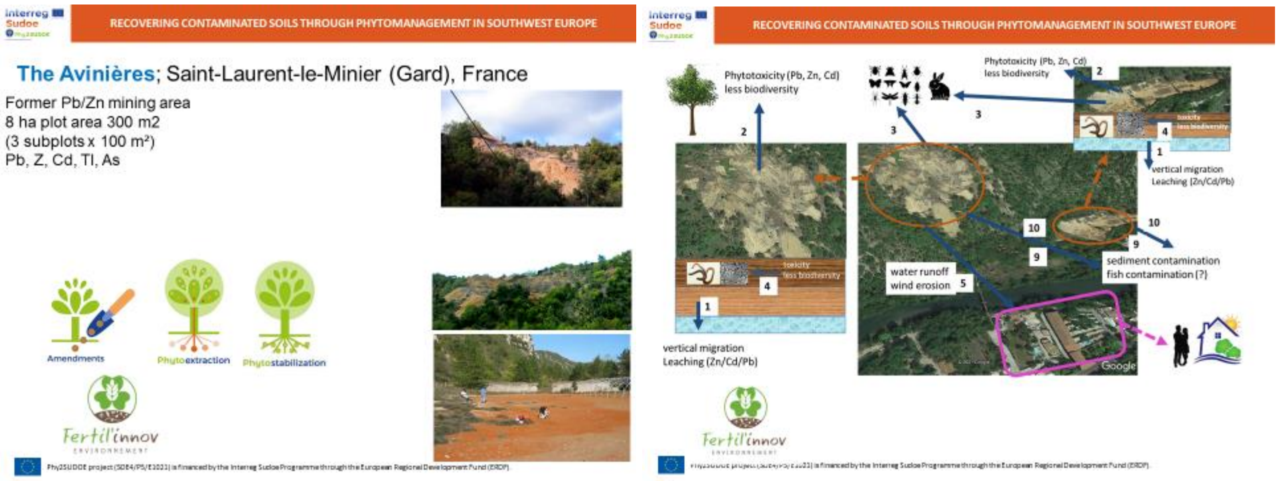
● **Implementation**:

The site has been subdivided into several zones with high metal concentrations. There are two areas with little or no vegetation: the slag heap on the hillside, where the old mining galleries can still be seen, and, at the bottom of the valley, the ore processing workshops and the old mining ponds on the left bank of the Vis. Both areas have a desert-like appearance due to the almost total absence of vegetation, and are extremely polluted with mining waste very rich in Zn, Cd and Pb. The heavy metal content in the former mining area is very high.

The risk of contamination of humans by ingestion or direct contact is very high. Wind erosion brings metal-laden dust into houses. During the rainy season, when small puddles are created, contaminants can pass from water to wild animals (deer, foxes, small mammals, amphibians, reptiles, etc.) by ingestion of grass or run-off water. It is also possible that pollutants migrate into water through runoff/sedimentation.

ADEME has been mandated to carry out safety work at the former mine. The objectives are as follows:

- Soil profiling by earthworks, stabilization of two gullies.
- Implementation of the phytostabilisation programme.
- Creation of an irrigation system for the plantations (including the necessary water reserves during the summer).



Location, map and conceptual model of Les Avinières site © Fertil'innov Environnement

● Soil properties

The study area is based on a limestone bedrock, the site of major tectonic accidents on the left bank slope. It is in these accidents (faults, crushed zones) that the ore is abundant, and where it was exploited in the open-cast mine of Avinières.

The waste rock of the former Avinières open-cast mine constitute a rather disorderly set of cuttings, often in the form of cones of scree with steep slopes, some located immediately downstream of mining galleries and others more likely related to surface mining. On the outskirts, they flow locally onto the old agricultural terraces.

These dumps are made up of blocks, pebbles and gravels which are essentially dolomitic (the bedrock of the ore mined), but which may contain a relatively large residual portion of ore. In particular, the finer sediments found on flat areas and under coarser material have high metal contents.

The metal(loid) contents in the former mining area are in excess. For the mining waste rock sector, these total soil contents ranged from 7 to 3300 mg As/ kg DM, from 22 to 1200 mg Cd/ kg DM, from 0 to 0.22 mg Hg / kg DM, from 3,800 to 50,000 mg Pb/ kg DM, 12 to 83 mg Tl / kg DM, and 41,000 to 54,000 mg Zn / kg DM. The pH of the substrate, being basic (> 7), arsenic is not very bioavailable for plants.

Anthropogenic soils developed on the Avinières site in heterogeneous materials of sandy-gravelly texture, carbonated with dolomite contents vary greatly over short distances (cf. layers C1 and C2, Fig. NS2. 4). The presence of several dark layers suggests different periods of pedogenesis (Sol II) linked to anthropogenic activity. The soil is colonized by short herbaceous vegetation, including a hyperaccumulator species, *Noccaea caerulescens*.

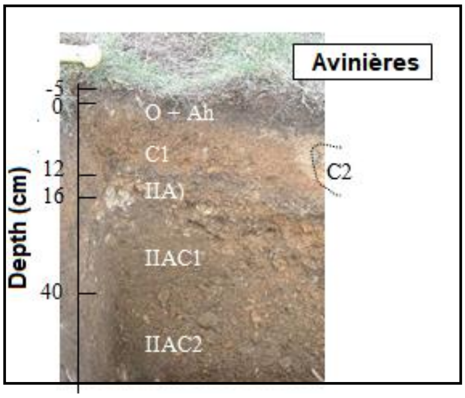


Figure NS2.1: Photographs of soils sampled on mine cuttings at the Avinières site

● Soil characteristics: organization and composition

Optical microscopy of thin sections taken from the O + Ah horizon of the Avinières mine soil (Fig. NS2.5a) shows that the first few centimeters are composed almost exclusively of plant debris, the cellular structures of which are usually still recognizable. The frequent presence of fungal hyphae indicates decomposition of this organic debris by fungi, but the absence of fecal pellets indicates little faunal activity. Gradually, the plant debris diminishes in size, the cellular structures become less recognizable, and in the Ah horizon (Fig. NS2.5b), it is predominantly black, angular organic matter. Fecal pellets are still absent, and consequently there is very little mixing of mineral and organic constituents in this Ah horizon, linked to very little faunal activity. The contact between the organic horizons and the mineral C1 horizon is remarkably abrupt and regular. These observations are very similar to those made in the holorganic horizon of the soil surface under metallic grassland (Balabane et al., 1999; Dahmani Muller et al., 2000; van Oort et al., 2002, 2007, 2008).

In the C1 horizon of the AV soil, the soil matrix is dominated by coarse skeletal particles, grains of weathering dolomite (Fig. NS2. 5c), juxtaposed with organic fragments. The plasma is made up of clay particles associated with iron oxyhydroxides that appear as coatings (ferrans), enveloping the mineral and organic constituents. The omnipresence of fine iron particles gives the soil a reddish-orange color. Electron microprobe analyses indicate the presence of Zn and Pb in these ferruginous coatings, in the order of 2% for each element. The various rock samples enable us to identify the original phases of the metals in the dolomite and the various stages of alteration. In the unweathered zones of the dolomite, numerous opaque, cubic crystals are present (Fig. NS2.7c), whose microprobe analysis confirms a PbS (galena) or ZnS (blende) composition. Alteration of the dolomite reveals characteristic spiral growth structures (Fig. NS2.5d). It generally begins around sulfide grains, whose oxidative alteration produces sulfuric acid, whose acidity is neutralized by Ca to produce gypsum. In the vicinity of alteration zones, Pb carbonates (cerussite), Zn carbonates (smithsonite) and mixed Fe, Mn and Pb sulfates (plumbojarosite) are found.

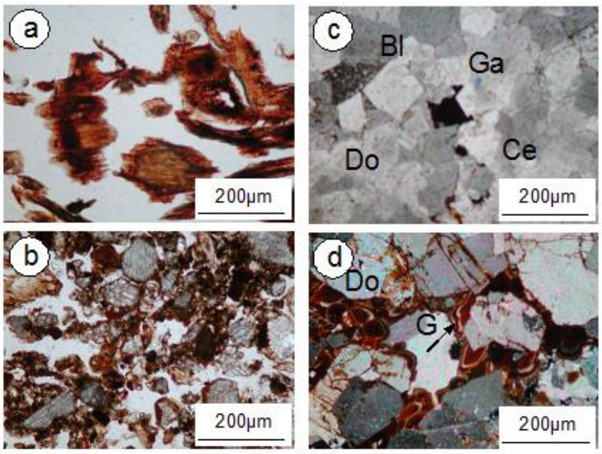


Figure NS2.2: Thin-slice optical microscopy images of Avinières (a-d) soil.

(a) organic fragments, O horizon, (b) juxtaposition between mineral and organic constituents, C1 horizon, (c) initial forms of metalliferous phases (Ga: galena, B: blende, Ce: cerussite, Do: dolomite), rock horizon, (d) altered rock, goethite formation.

● **Chemical and physicochemical soil properties**

Texture, carbonate content, pH and CEC. Generally speaking, soils developed on mine cuttings are characterized by a grain size dominated by sandy fractions, reflecting the short weathering time of these mineral materials (only a few decades to a few hundred years), as well as the presence of weathering dolomite, a process that always leads to a very sandy material. The Avinières soil horizons contain variable amounts of dolomite, ranging from 3 to 23% (and up to 75% in the C2 horizon). They show pH-water values between 7 and 8. The exchange complex is largely dominated by Ca and Mg, in keeping with the dolomitic nature of the surrounding geological materials. In the Petra Alba samples, the fraction < 20µm (clay and fine silts) is more important, in line with the schistose nature of the excavated material. The soil contains only traces of carbonate (< 0.2%) and is weakly acidic (pH 6.3 to 6.4) (Table NS2.2).

CEC values are high, due to the presence of around 20% clay and significant quantities of OM. The materials in the settling basin are clearly distinguishable from the other two soils, with a grain size dominated by the clay fraction (> 50%) and the virtual absence of particles > 50µm. The sandier-textured surface layer is largely influenced by colluvial materials from the slopes.

Organic matter. In the Avinières soil, organic carbon content is around 1 to 2% in horizons IIA - II AC2 (Table NS2.2, Fig. NS2.4). The value of the C/N ratio can be an indicator of pedogenic intensity (Néel, 2003), but where metals are present, they can have a slowing effect on OM degradation, leading to high C/N values. These C/N values are very high, between 20 and 45, with a value of 14 observed in the horizon interpreted in the field as the old surface horizon (IIA). The value of 20 observed in the litter horizon of the metalliferous species vegetation is compatible with those observed in a metalliferous lawn under *Arabidopsis halleri* and *Armeria maritima* ssp *halleri* vegetation (Balabane et al., 1999; Dahmani-Muller et al., 2000). Values of around 40 could be explained by the presence of fine coal particles, resulting from in situ roasting of ores. The value of 14 in horizon IIA would be compatible with the presence of a former surface horizon of temporary soil under vegetation, covered by around 15 cm of material when the site was abandoned.

Organic matter accumulation. In the Avinières soils, the accumulation of organic matter on the surface is remarkable: it takes the form of a 5 to 7 cm thick layer of litter, made up of plant debris whose cellular structures are usually still clearly recognizable. Fungal hyphae are frequently present, indicating fungal decomposition, but the absence of fecal pellets indicates low faunal activity. The incorporation of OM into the soil therefore appears to be very limited, an aspect very similar to observations made in the case of ecosystems heavily polluted by metals: metal-bearing lawns (Van Haluwyn, 1987; Balabane et al., 1999; Dahmani-Muller et al., 2000; van Oort et al., 2002a).

Table NS2.1: Main physico-chemical properties of Les Avinières soil horizons.

		Granulometry, g.kg^{-1}						MO				Exchangeable cations	
Horizon	Depth (cm)	Clay	Fine silts	Coarse silt	Fine sands	Coarse sands	$CaCO_3$ g.kg^{-1}	C org. g.kg^{-1}	C/N	pH	CEC	Ca^{2+}	Mg^{2+}
											cmol^{+} kg^{-1}		
Avinières													
Ol+Ah	+5 - 0	153	138	117	181	411	230	129	20.4	7.1	13.7	9.8	3.5
C1	0 - 12	290	139	76	165	330	69.3	31.1	41.3	7.2	5.2	3.0	1.9
C2	-	*61*	*43*	*53*	*243*	*600*	*749*	*4.05*	*14.0*	*7.5*	*1.4*	*0.8*	*0.5*
IIA	12 - 16	331	191	123	216	139	29.9	11.2	15.3	7.5	9.9	6.1	3.4
IIAC1	16 - 40	269	166	119	212	234	188	24.6	44.2	7,9	8.1	4.9	2.9
IIAC2	> 40	283	189	135	192	201	136	15.2	25.3	8.0	9.7	6.0	3.4
Settling basin													
- 1	0 - 10	89	193	307	334	77	108	51.6	90	7.1	0.72	0.54	0.09
- 2	10 - 20	563	353	79	4	1	89	12.8	31.5	7.0	7.0	5.5	1.18

In conclusion, the study of soils developed on mine cuttings dating from different periods of mining (> a century for the Avinières site) has highlighted the heterogeneity of the parent materials, differences in their nature, metal contents, and differences in speciation (metals included in the crystallographic structures at Avinières).

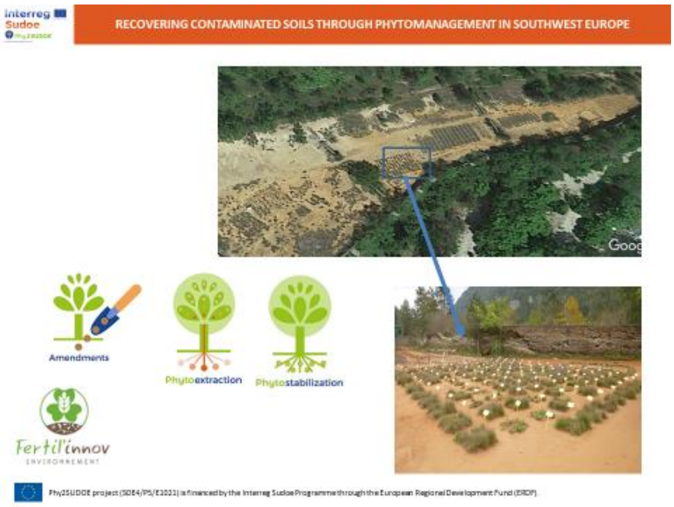
- **Phytomanagement options /plant assembly**

The revegetation of polluted sites in the South of France should be based on indigenous species that are tolerant to high metal(loid) concentrations. In addition to their metal(loid) excess, these sites are characterized by a high aridity level, with the summer drought typical of the Mediterranean region exacerbated by a coarse, filtering substrate (Escarré et al., 2000). It is therefore reasonable to assume that, on these sites, it must be difficult to establish plants used for phytoremediation in more northerly regions with a temperate climate and no pronounced summer drought. Plant species that could potentially be used for revegetation must be fast-growing, cover well and be able to develop on the different sites. The use of species from the Fabaceae family is essential to bring nitrogen nutrition to soils that are both phytotoxic and nutrient-poor. The plant species used must be primarily metal(loid) excluders so as not to be toxic for the herbivory present on the site. In this respect, we showed that herbivorous animals are not able to avoid plant species with high metal content. These results refute the hypothesis that the metal(loid) hyperaccumulation protects plant species from herbivores.
Finally, we presented the results of a phytoremediation experiment using certain plant species present on the three sites with high metal(loid) levels and capable of developing a dense plant cover that is stable over time.

Several plant species have adapted to the soil and climatic conditions of the Avinières site and, despite the constraint of high levels of metal(loid)s, a significant diversity of plants can be observed. The following plant species are mainly observed: *Armeria arenaria, Noccaea caerulescens, Festuca arvernensis, Koeleria vallesiana, Biscutella laevigata, Anthyllis vulneraria, Lotus corniculatus, Silene vulgaris, Reseda lutea, Plantago lanceolata* and, more scarcely, *Alyssum montanum.*

The objective is to phytostabilize the soil and to sustainably cover the contaminated soils by excluder plants. In this context, some pilot tests have been carried out with the following treatments:
- Soil treatments: compost amendment.
- Plant treatment: Mixture of metal tolerant herbaceous species.
- Biological treatments: Inoculation with symbiotic bacteria and mycorrhizal fungi.

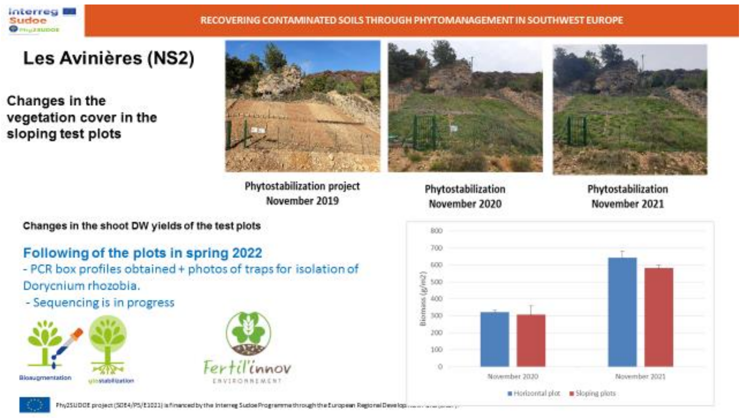
In situ tests were set up at the end of 2019 before proceeding with the rehabilitation of the old mine to confirm the effectiveness of the supply of organic matter and optimize the association of the various plant and microbial species. The pilot tests made it possible to define the phytostabilization protocol for Les Avinières:

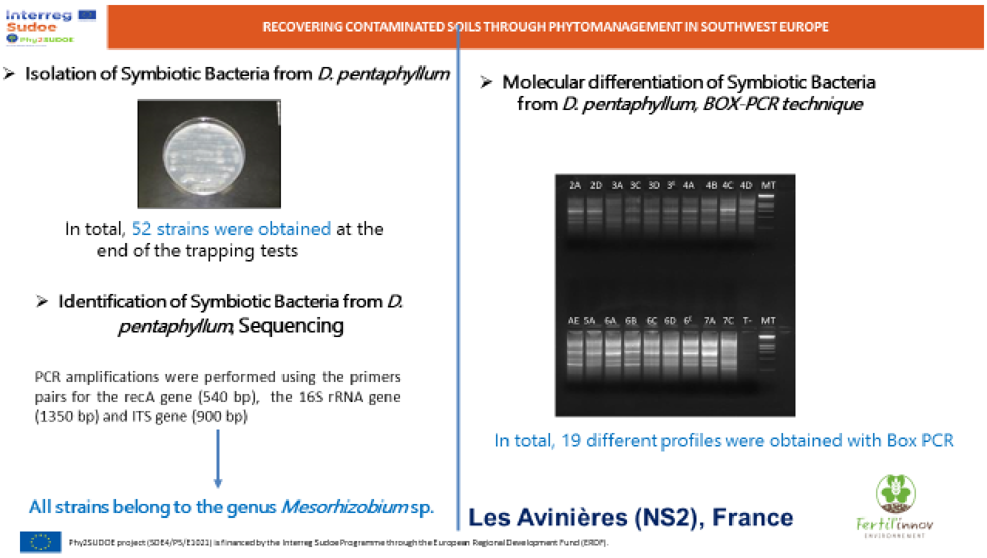


Focus on field plots at Les Avinières site © Fertil'innov Environnement

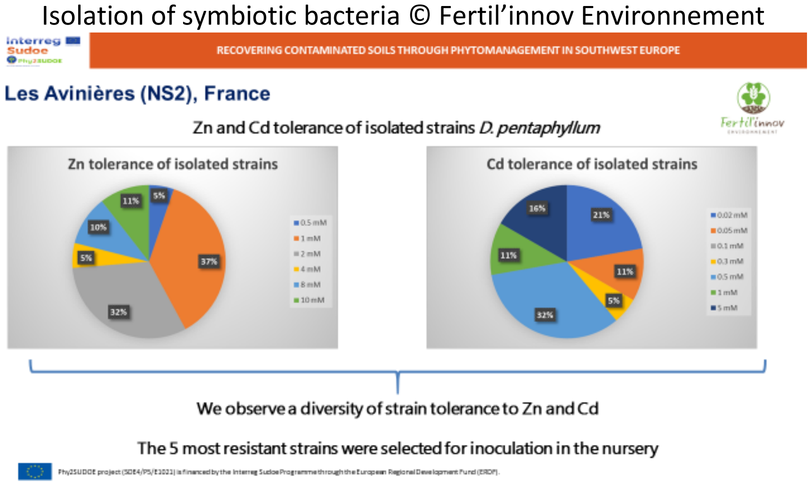
● **Success / limits:**
- Changes in the vegetation cover and the shoot dry weight yields were recorded in the field plots. The dense and perennial plant cover limits erosion: only metal-tolerant species are able to grow on the Avinières substrate. More than 10 species were retained and the monitoring of metal levels in the aerial parts shows that the species selected for phytostabilization are not hyperaccumulating species. Recovery rates are very satisfactory (>95%).
- The monitoring of the fluorescence at the level of the leaves shows a satisfactory level and indicates a good state of greenness of the plants.
- The isolation and characterization of symbiotic bacteria were carried out on the nodules formed on the roots of *Dorycnium pentaphyllum* (a Fabaceae). 52 strains belonging to the *Mesorhizobium* family were selected and metal resistance tests were subsequently carried out. Five strains resistant to zinc and cadmium were selected to inoculate *Dorycnium* seedlings in the nursery. After development of the root system and the installation of nodulation, the plants were planted in autumn 2022 on Les Avinières substrate.
- The content of organic matter, total nitrogen and microbial biomass of soil are improved: the plant cover enriches the environment with nitrogen and organic matter.
- A diversity of microorganisms beneficial to the growth of plants is more important under the plant cover: the analysis of the diversity in the soil after 1 year of cover shows that compared to the initial soil, a strong presence of the Ascomycota, Basidiomycota and Mortierellomycota Phylum is recorded, which appears as a potential marker of vegetated soil.

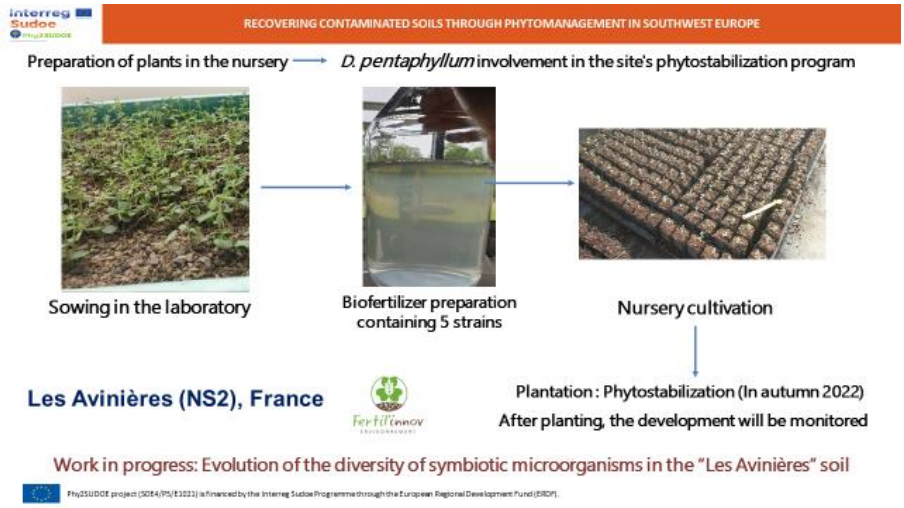
The Avinières site was revegetated in 2022 and the monitoring of the vegetation cover is continuing.
The pilot tests demonstrated that it is possible to achieve the site phytostabilization while accounting for several parameters: the soil, the climate, the plant species and the microorganisms.





Isolation of symbiotic bacteria © Fertil'innov Environnement





Dorycnium pentaphyllum and rhizobia nodules (© S. Soussou, Fertil'innov Environnement)

Analysis of plant community. At the soil sampling plots, vegetation surveys were carried out over an area of 100 m^2. The layout varied from a square (10 x 10 m) to a rectangle (5 x 20 m), depending on the shape of the vegetation zone analyzed. All plant species present were recorded.
Around 116 species were recorded on and around the Avinières site. The matrix of plant species and records was subjected to multivariate analysis to position the plant species in relation to each other.
In addition to presence-absence data on the plant species present at each plot, data on the mineral content of the soil at each plot (As, Ca, Cd, Fe, K, Mg, Mn, P, Pb, Tl, and Zn) were also taken into account. Only plant species present at least 3 times are included in the calculations.

Axis 1 of the factorial correspondence analysis (Fig. NS2.3) allows to distinguish between metal(loid)-tolerant plant species (located at the top left of the graph) and non-tolerant plant species present on relatively unpolluted soils (right-hand side of the graph) (Escarré et al.2011). The plant species present on the least polluted sites include *Aphyllantes monspeliensis*, *Thymus vulgaris, Scabiosa maritima, Plantago lanceolata* and *Bromus erectus*. These species, unlike tolerant species which are absent or scarce outside polluted sites, are present on the periphery of the site and are common in the region. They disappear from sites as metal(loid) levels rise. Tolerant plant species include *Festuca arvernensis, Koeleria valesiana* and *Anthyllis vulneraria*. Only Anthyllis is present on the Avinières site. Other plant species such as *Armeria arenaria, Jasione montana, Noccaea caerulescens, Biscutella laevigata, Helianthemum nummularium* and *Iberis intermedia* are also present on all polluted sites. The surveys identified a Fabaceae species, *Genista pilosa,* which unlike *A. vulneraria*, is fairly common on the region's mining sites.

Most of the observations made show a depletion of plant species on sites where the substrate or soil has a high metal(loid) content. The extreme case is illustrated by the surveys along the edges of the settling ponds, where only the most tolerant plant species are found, such as *Noccaea caerulescens, Armeria arenaria, Festuca arvernensis* and *Koeleria valesiana*.

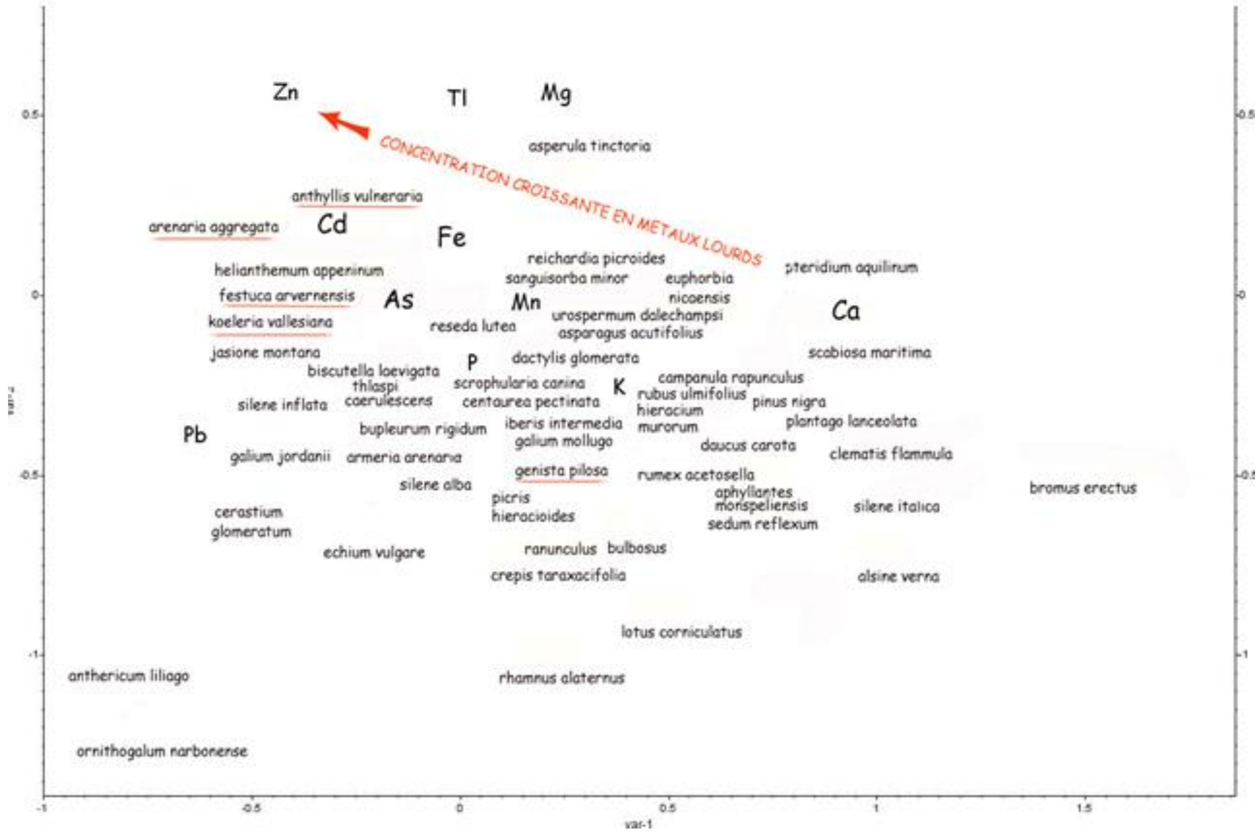


Figure NS2.3: Correspondence factor analysis of vegetation and soils at the Avinières site.

Phytostabilization experiments in the Avinières settling basins.

On the basis of initial floristic surveys, an experiment was carried out in settling basin no. 1 with the aim of assessing the potential of plant species present on the St-Laurent-Le-Minier metalliferous sites, in terms of cover, stability and persistence.

The plant species selected were two *Poaceae* (*Koeleria vallesiana and Festuca arvernensis*), a *Plumbaginaceae (Armeria arenaria*) and a Fabaceae, *Anthyllis vulneraria*. The latter was chosen for its potential to fix atmospheric nitrogen and enrich the soil with nitrogen, despite its ability to accumulate metals. As this species is monocarpic (practically all individuals disappear after flowering), it must be reseeded in order to persist. The 4 species selected were divided into 15 different associations in 3 blocks totaling 45 plots. The layout of the experiment is shown in figure NS2. 7. Each plot could contain mixtures of one, two, three or four species. Each plot had 6 x 6 plants, but only the 16 plants on the inside were harvested. Prior to planting, horse compost (30% organic matter, 1% nitrogen pH=7.0) was incorporated into the mineral substrate at a rate of 1.5 Kg/m^2.

For two years, non-destructive monitoring of vegetative and reproductive biomass, mortality and cover was carried out for each species and plot. This monitoring was based on allometric relationships between biomass and the number, length and width of leaves. This relationship was obtained after harvesting plants present on the site but not used in the experiment. After two years, two plants per species and per plot were harvested for analysis of biomass and mineral element content. Two soil cores were taken from each plot, and the nitrogen content of these samples was measured. All results are published in Frérot et al (2006).

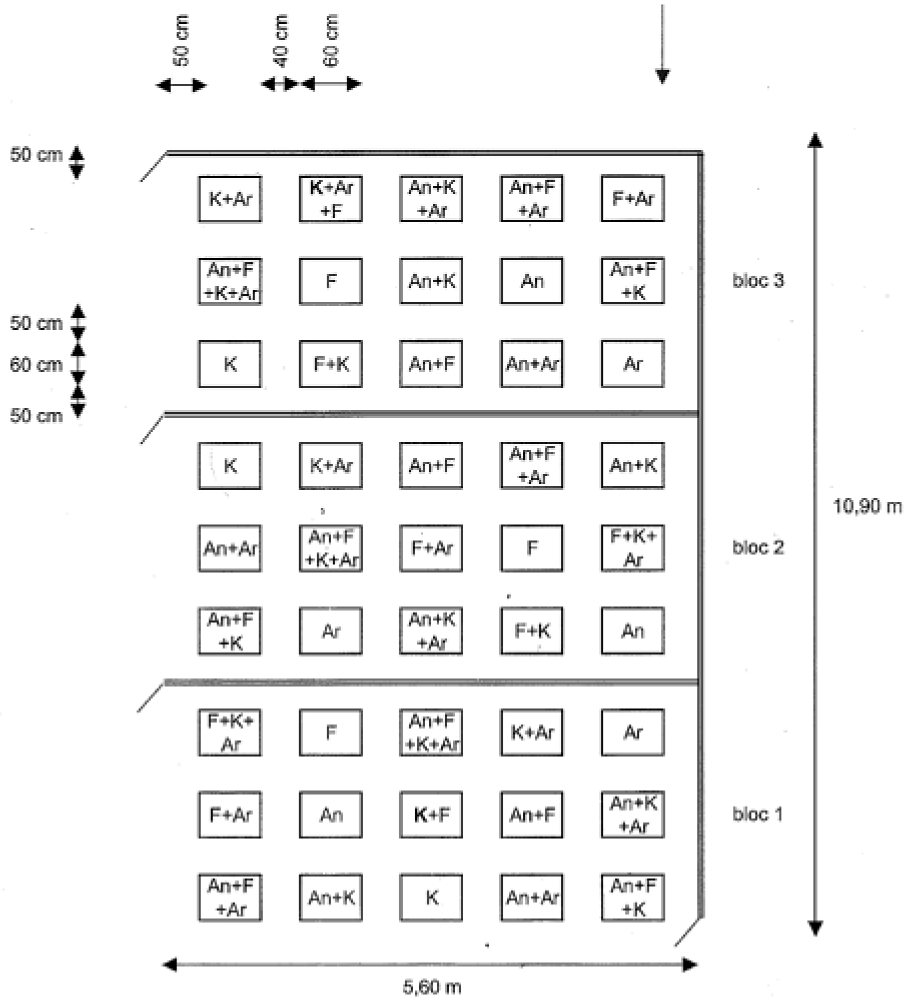


Figure NS2. 4: Layout of phytoremediation experiment.
Three blocks of 15 plots each contained different combinations of species, with one, two, three or four species per plot. In each plot 6 X 6 individuals were planted. An= Anthyllis vulneraria; Ar=Armeria arenaria; F= Festuca arvernensis; K= Koeleria vallesiana. Figure taken from Frérot et al (2006).

Plot coverage results after five years are shown in Fig. NS2.8.

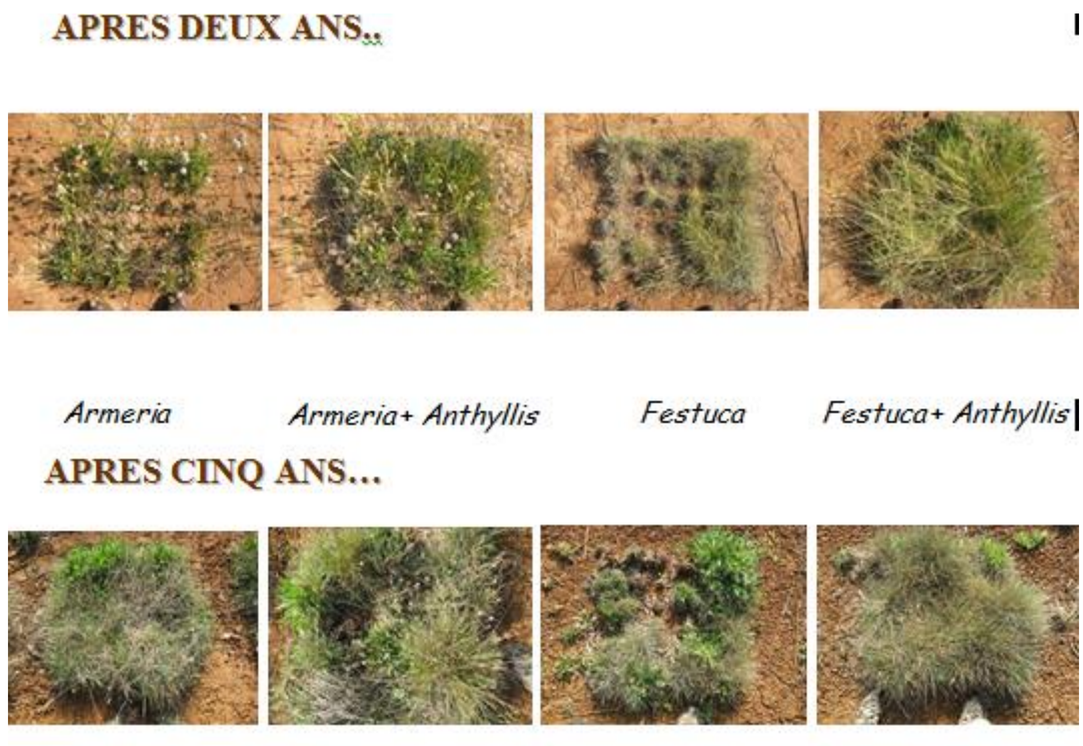


Figure NS2.5: Pics of selected plots taken two and five years after the start of the experiment.

Interest of the *A. vulneraria* /*M. metallidurans* association: metal tolerance and rhizodeposition

For assessing the adaptation degree of different *Anthyllis* ecotypes or subspecies to the presence of high metal(loid) concentrations, field surveys identified several *Anthyllis vulneraria* subspecies suitable for phytoremediation (Mahieu et al., 2011). A study was carried out on four populations, two from former Zn-Pb mines (MET), Avinières in the Gard ([Zn] = 26000 mg kg^{-1}) and Eylie en Arriège ([Zn] = 4632 mg kg^{-1}) and two from uncontaminated soils (NMET) in the Causses.

Each population was cultivated both in its original soil and in those of the other populations. The NMET populations showed high mortality and low growth rates in highly contaminated soils, whereas the Avinières plants showed high growth rates in soils with high metal(loid) levels. Individuals from Avinières seem well suited to the remediation of highly contaminated sites (> 30000 mg Zn kg^{-1}) while those from Eylie could be used for the remediation of less contaminated soils (<30000 mg Zn kg^{-1}).

Given the wide distribution of *A. vulneraria*, it is entirely conceivable that both populations could be used to restore abandoned mining sites in the temperate regions of Southern and Northern Europe (Mahieu et al., 2013).

Beyond the identification of plant species and microorganisms of interest for phytostabilization, the analysis of plant-bacteria interactions revealed a very positive effect of bacteria, which contribute to decreasing the metal content (zinc and cadmium) in the aerial parts of *A. vulneraria* subsp carpatica (subspecies present on the Avinières site) (Fig. NS2.9). In parallel, a protective effect of the symbiotic metallicolous bacterium (Vidal et al., 2009) has been observed on non-metallicolous *Anthyllis* ecotypes (improved tolerance to metal stress) (Soussou et al., 2013).

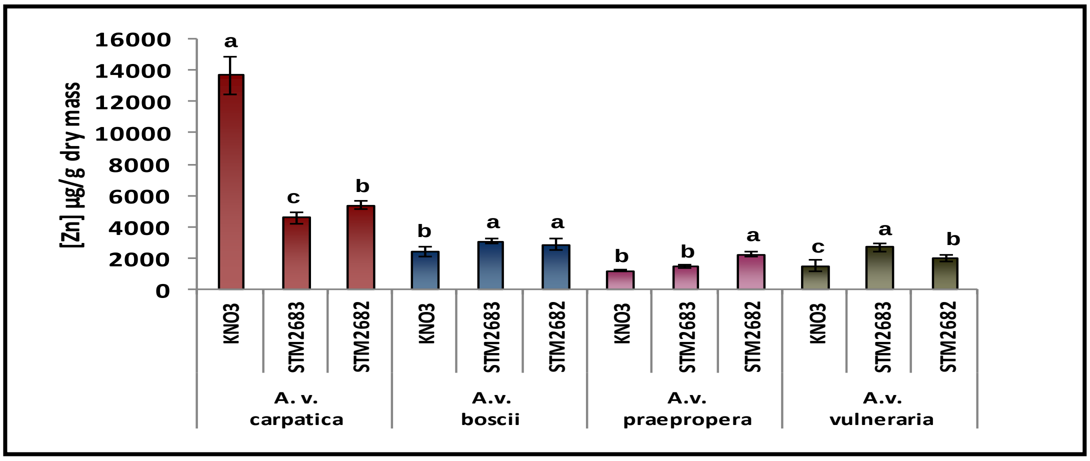


Figure NS2. 6: Zinc concentration in aerial parts of plants exposed to 1000 µM Zn

In addition, *A. vulneraria* subspecies *carpatica* and its symbiotic bacterium, *Mesorhizobium metallidurans*, have been shown to increase the N content of mine tailings in the soil, due to their ability to develop a strong nitrogen-fixing potential (Mahieu et al., 2014). The presence of *Anthyllis*, by favoring the entry of nitrogen, facilitates real vegetation dynamics with the installation of new plant species such as *Festuca* and *Koelleria* (Frérot et al., 2006).

Conclusion

The use of plants is increasingly being considered as a means of completing the redevelopment of sites impacted by metal(loid) excess. Feedback from experiments confirms the potential of phytomanagement, but the fact remains that each site, due to the history of its operation and the metal(loid) content of the substrates or mine tailings, is a specific case study. Before a vegetation cover can be installed on a long-term basis, the conditions under which the plants will develop must be carefully controlled (Bert et al., 2017). Among the many factors to be taken into account, the bioavailable metal(loid) and organic matter levels in the substrate are imperative, as they condition the success of a redevelopment involving a phytostabilization operation.
If bioavailable metal(loid) levels are low, it is possible to use a fairly wide range of plant species when sufficient corrections have been made to the substrate (pH, organic matter). On the other hand, when the bioavailable metal(loid) content of the substrate or mine tailings is high, it is imperative to consider the vegetation that spontaneously colonizes the contaminated environment, in addition to improving its physico-chemical properties.

In the case of the former Avinières mine, we have solid scientific data on the nature of the substrate, the vegetation and the spontaneous soil microflora adapted to the high content of the mining substrate. Experiments carried out *in situ* have demonstrated the full potential of plant and plant-microorganism associations. The choice of plant species, their multiplication and the corrections made to the substrate will be decisive in the rehabilitation of the Avinières mine site.

Field trial - Initial state of the Avinières substrates

Soil samples were taken from both vegetated and bare areas. In each area, several horizons were observed: 0-10 cm surface horizon, 10-20 cm horizon and 20-30 cm horizon (deep substrate).

Granulometry. Granulometry was determined for each horizon in order to establish the proportion of fine elements, i.e. those most immediately reactive in soil reconstitution (elements smaller than 2 mm).

In the bare zones, the fine fraction (< 2 mm) of the 0-10 and 10-20 cm horizons exceeds 60% (Figure NS2.15&16), while in the 20-30 horizon this fraction does not exceed 35%. However, in the mulched substrate (vegetated areas), the fine fraction is lower: 51% and 39% respectively for horizons 0-10 and 10-20 cm, and 31% for horizon 20-30 cm (Figure NS2.14&15).

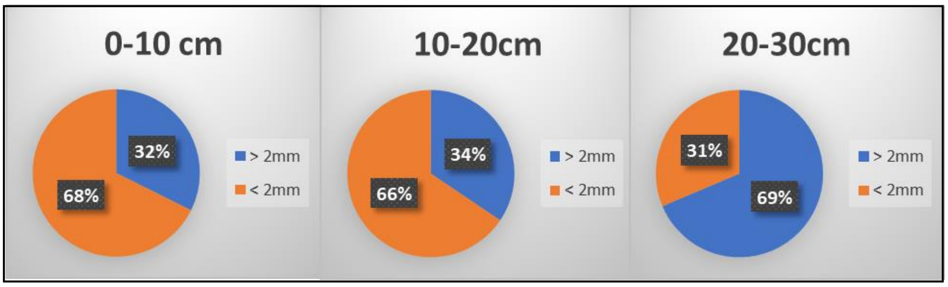


Figure NS2.7: Overall grain size of the 0-10 cm, 10-20 cm and 20-30 cm horizons of bare soil

Figure NS2.8: Overall grain size of the 0-10 cm, 10-20 cm and 20-30 cm horizons of a vegetated soil

Physico-chemical analysis. All physico-chemical analyses were carried out on the fine fraction (< 2 mm).

All the horizons analyzed are characterized by a sandy texture.

All horizons have a basic pH, negative Eh values and contain significant quantities of total limestone and magnesium oxide. All profiles show pronounced phosphoric anhydride and potash deficiencies, and are characterized by low Cation Exchange Capacity (CEC) (Table NS2.3).

Table NS2.2: Main physico-chemical properties of Les Avinières soil horizons.

Site	Echantillons	Argiles (pour mille)	Limons fins (pour mille)	Limons grossiers (pour mille)	Sables fins (pour mille)	Sables grossiers (pour mille)	Matière organique (%)	Azote total (%)	Rapport C/N	pH eau	Eh	Calcaire total (g/kg)	Calcaire actif (g/kg)	CaO (g/kg)	CEC (cmol+/kg)	P_2O_5 (g/kg) Méthode Joret Hébert	K_2O (g/kg)	MgO (g/kg)
Substrat nu	0-10 cm	39	37	94	349	481	0,6	0,029	27,3	7,9	-58,8	117	1	0,5	2,5	0,03	0,021	0,097
	10-20 cm	96	25	96	296	486	0,4	0,028	17,1	7,7	-52,7	157	<1	<0,5	2,7	0,059	0,019	0,104
	20-30 cm	66	47	90	267	530	0,13	0,025	32,6	8	-60,7	143	1	0,58	2	0,03	0,03	0,161
Substrat végétalisé	0-10 cm	35	42	91	398	435	2,2	0,058	12,5	7,9	-58,8	121	<1	0,92	1,9	0,03	0,025	0,112
	10-20 cm	47	16	107	564	266	1,9	0,031	17,1	8	-61,7	136	<1	0,68	1,8	0,03	0,025	0,09
	20-30 cm	53	68	123	365	392	1,1	0,027	24,8	8	-62,9	129	<1	0,56	2,3	0,03	0,022	0,133

Humic status. The parameters measured were organic matter content (g/kg), total nitrogen (g/kg) and C/N ratio for the different horizons.

Organic matter levels are very low. All the samples analyzed showed deficiencies in total nitrogen and very high C/N ratios (Table NS2.3). However, the superficial horizon (0-10 cm) and, to a lesser extent, the 10-20 cm horizon of the vegetated zone contain more total nitrogen, and the C/N ratio is close to desirable levels in a fertile soil...

Metal(loid)s. Metal(loid)s were analyzed in the various horizons. Their total concentrations were high in all soil samples tested. However, they were slightly lower in the vegetated soil horizons (Table 4).

Table NS2.3: Metal(loid)s analyzed by MP-AES in the horizons of bare and vegetated soils.

Soil	Horizon	Cd	Zn	Cu	Pb	As	Tl	Fe	Mg
Bare soil	0-10	118,9	26 442,3	43,6	30 869,8	61,2	60,9	37 953,1	3 745,4
	10-20	628,2	56 769,9	46,0	41 209,9	424,4	70,8	42 021,9	3 552,9
	20-30	528,2	53 769,9	43,1	40 209,8	424,4	79,9	41 521,8	3 132,9
Soil under vegetation	0-10	86,9	16 536,7	40,1	16 048,9	394,4	41,0	35 258,3	4 979,7
	10-20	136,0	22 644,6	40,6	21 844,4	258,1	36,0	28 285,7	5 019,4
	20-30	128,0	23 918,3	27,8	9 905,7	279,5	67,0	36 667,6	4 536,0

Figure NS2.9: Pics of plant species identified at the Avinières site

- **Conclusion 1**

The *in-situ* diagnosis shows that the physico-chemical properties of the Avinières substrate are low in organic matter and markedly deficient in nitrogen, phosphoric acid and potassium.

The study of the vegetated zones highlighted the following points:

- Lower metal(loid) content in the upper horizons

- Slightly higher N content in vegetated soils
- Better water infiltration when the mining substrate is either vegetated or has a high organic matter content.

Metallicolous plant management

A number of plant species have adapted to the soil and climate conditions at the Les Avinières site, despite the constraints imposed by the high metal(loid) levels, and a significant diversity of plant species can be observed in the area where the former mine was operated.

The following plant species were recorded during *in situ* diagnostics: *Koeleria vallesiana, Armeria arenaria, Festuca arvernensis, Anthyllis vulneraria, Arenaria agregata, Biscutella laevigata, Sanguisorba minor, Centaurea pectinata, Dactylis glomerata, Lotus corniculatus, Reseda lutea, Silene latifolia, Plantago lanceolata, Cervaria rivini, Genista Pilosa, Iberis sp., Poa sp. Noccaea caerulescens,* and *Euphorbia characias*. (Figure NS2.16).

Seeds of several species were harvested. Soil samples were also taken and stored at 5°C.

Soil treatments

In order to improve the soil fertility, the substrate was amended with compost.

Plant treatments

3 pilot tests have been set up: one horizontal plot of 100 m^2 and two sloping plots of 100m^2 each.

The pilot tests were carried out as part of the redevelopment program of zone F (Les Avinières) of the former site of the Saint Laurent le Minier mine (Gard) (Figure NS2.17).

Each plot test was divided into 4 sub-plots:

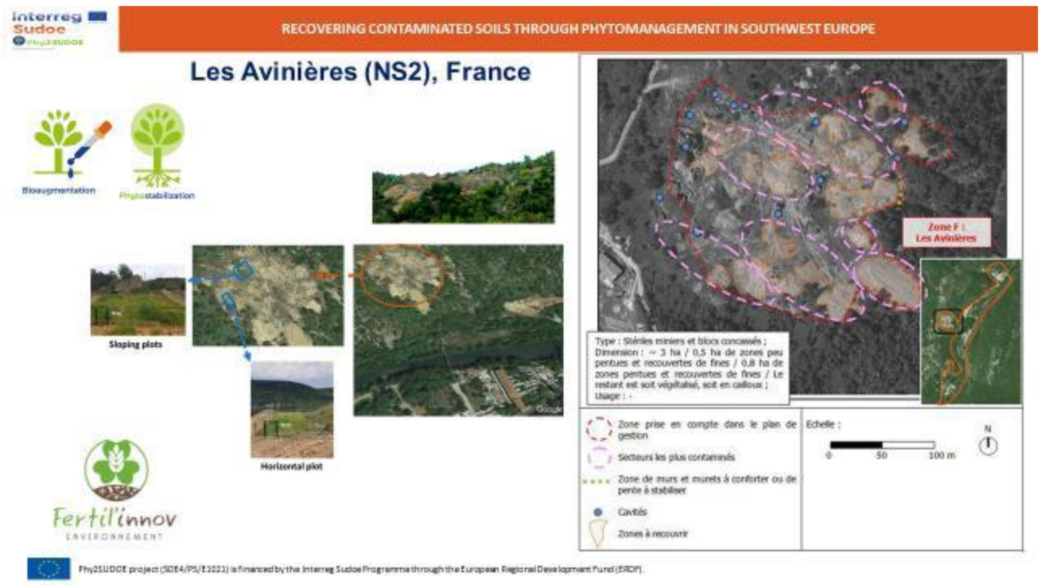
* Sub-plot N° 1 (25 m^2): Mixture of 100% metals tolerant species (seeds collected on the site): sowing;

* Sub-plot N° 2 (25 m^2): Mixture of 100% metals tolerant species (seeds collected on the site): sowing + planting;

* Sub-plot N° 3 (25 m^2): Mixture of 100% not metals tolerant species (commercial seeds): sowing;

* Sub-plot N° 4 (25 m^2): Mixture of 50% metals.

Biological treatments. Inoculation with symbiotic bacteria and mycorrhizal fungi.



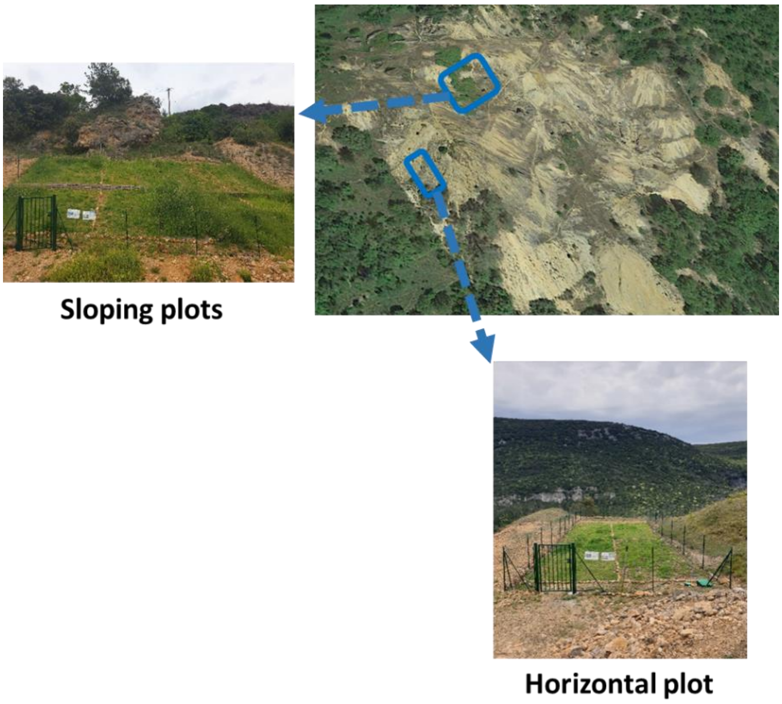


Figure NS2.10: Location of the field plots of Les Avinières site © Fertil'Innov Environnement

Isolation of symbiotic microorganisms

Plants of *Dorycnium* sp were cultivated in order to isolate the symbiotic bacteria associated with them. Rhizospheric soil was also sampled for trapping tests in the laboratory.

- Root observation: very few nodules were observed (because the sampling period was not optimal). Nodules were collected and stored at -80°C.
- Trapping trials were carried out under controlled conditions (Figure NS2.18). After 8 weeks of cultivation, the *Dorycnium* sp plants were harvested and the nodules removed. Isolations were made to obtain microbial cultures. The isolates obtained were tested on a culture medium containing Zn and Cd to verify their adaptation to metal excess (Figure NS2.19).

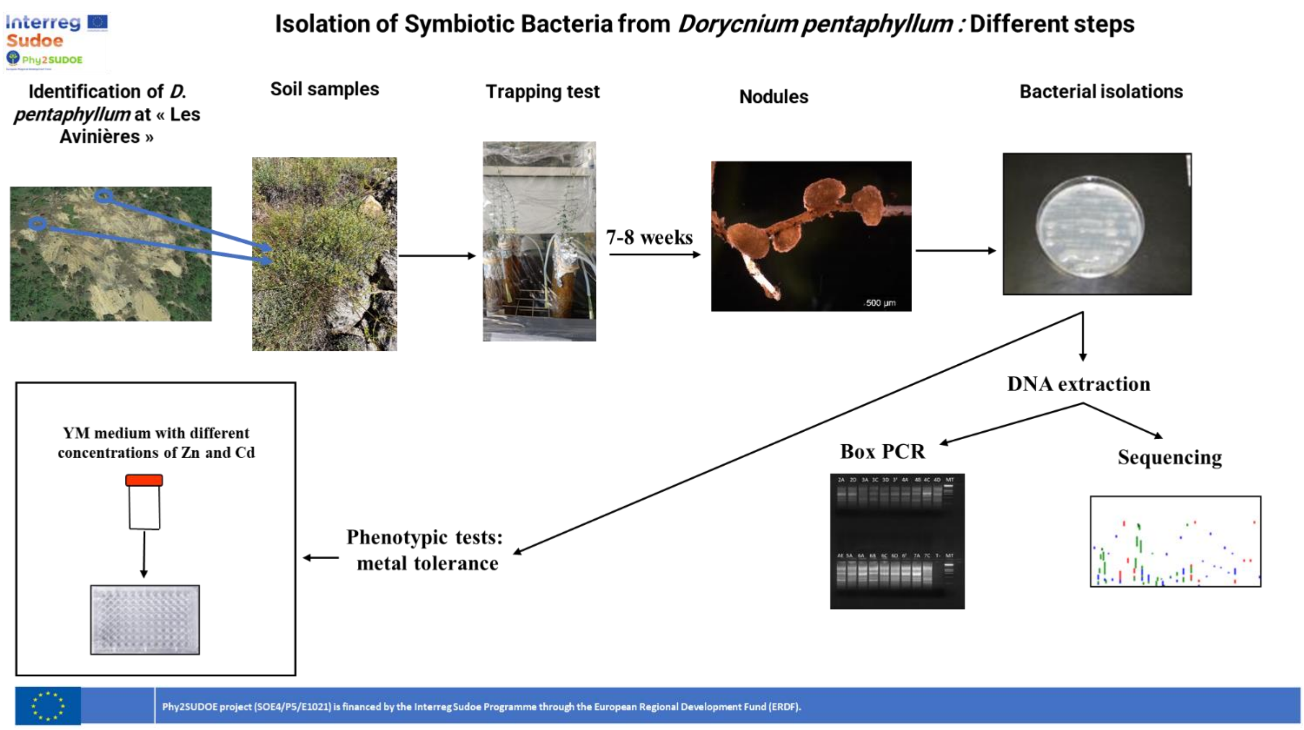


Figure NS2.11*:* Isolation of symbiotic bacteria from *Dorycnium pentaphyllum* : Different steps

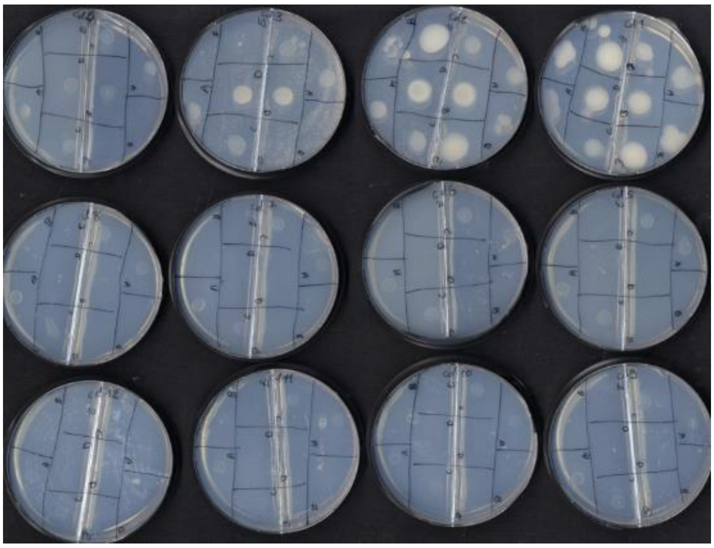


Figure NS2.12: Photo illustrating tolerance testing of different strains on solid medium in the presence of Zn

In addition, strain tolerance tests in liquid media were carried out (Figure NS2. 20).

Zn and Cd tolerance of isolated strains *D. pentaphyllum*

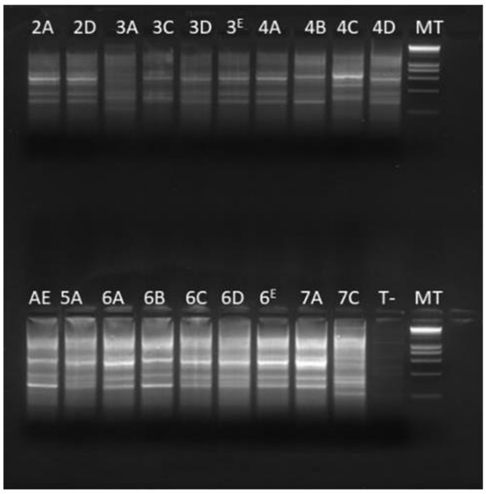
We observe a diversity of strain tolerance to Zn and Cd

Figure NS2.13: Zn and Cd tolerance of isolated *Dorycnium* strains

Molecular characterization

All isolated strains were identified. Box PCR was performed (Figure NS2.21).

➢ **Molecular differentiation of Symbiotic Bacteria from *D. pentaphyllum, BOX-PCR technique***



In total, 19 different profiles were obtained with Box PCR

Figure NS2. 14: Box PCR with strains of *Dorycnium* and PCR amplification was carried out using primers for the recA gene (540 bp), the 16S rRNA gene (1350 bp) and ITS gene (900 bp).

- All strains belong to the genus *Mesorhizobium* sp.

Physico-chemical analyzes

Changes in physico-chemical properties was monitored in the Avinières trial plots.

All physico-chemical analyzes focused on the fine fraction (<2 mm). The parameters measured are: granulometry, water pH, total limestone (g / kg), active limestone (g / kg), the P_20_5 content, the K_20 and MgO contents (g / kg), microbial biomass, total nitrogen and total carbon.

A raw substrate analysis was also included.

All samples were taken from 0-30 cm deep.

- The raw substrate from Les Avinières (initial state) has low organic matter, nitrogen and nutrient content. The microbial biomass is also very low (Table NS2.5, Figure NS2.22).
- Amendment of the substrate helped improve soil fertility (Figure NS2.22).
- The installation of plant cover (phytostabilisation tests) has a positive effect: Increase in the rate of organic matter, nitrogen content in the soil as well as microbial biomass (Figure NS2.22).
- We observed an increase of organic matter, total nitrogen and microbial biomass between the 2021 and 2023 monitoring (Table NS2.5, Figure NS2.22).

Table NS2.4**:** Physico-chemical analyzes

Samples	Depth	Clays		Fine silts		Coarse silts		Fine sands		Coarse sands		Organic matter (%)		Total nitrogen (g/kg)		pH water		total limestone (g/kg)		P_2O_5 (g/kg)		K_2O (g/kg)		MgO (g/kg)		Microbial biomass (mg/kg sec)	
Years		2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023	2021	2023
Raw substrate without vegetation	0-10 cm	40	39	36	37	100	100	340	337	484	487	0,5	0,5	0,52	0,52	8	7,8	121	121	0,011	0,011	0,019	0,019	0,033	0,031	48,31	48,31
Substrate + compost / Horizontal plot Without vegetation	0-30 cm	37	38	50	52	99	97	390	387	424	426	1,9	2	1,68	1,69	7,9	7,9	134	134	0,14	0,14	0,1	0,1	0,165	0,174	195,82	196,55
Substrate + compost / Sloping plots Without vegetation	0-30 cm	53	49	68	70	123	124	365	367	392	390	2	2,1	1,47	1,5	8	8	122	122	0,1	0,1	0,12	0,1	0,1	0,11	145,7	146
Substrate + compost / Horizontal plot With vegetation (phytostabilizsation)	0-30 cm	37	39	50	52	99	94	390	388	424	427	3,26	3,36	2,65	2,84	8	7,9	134	134	0,1	0,17	0,16	0,18	0,23	0,25	519,29	522,11
Substrate + compost / Sloping plot With vegetation (phytostabilization)	0-30 cm	46	50	17	17	105	105	568	560	264	268	3,15	3,23	2,36	2,59	8	8	130	130	0,1	0,18	0,2	0,25	0,13	0,19	449,06	452,3

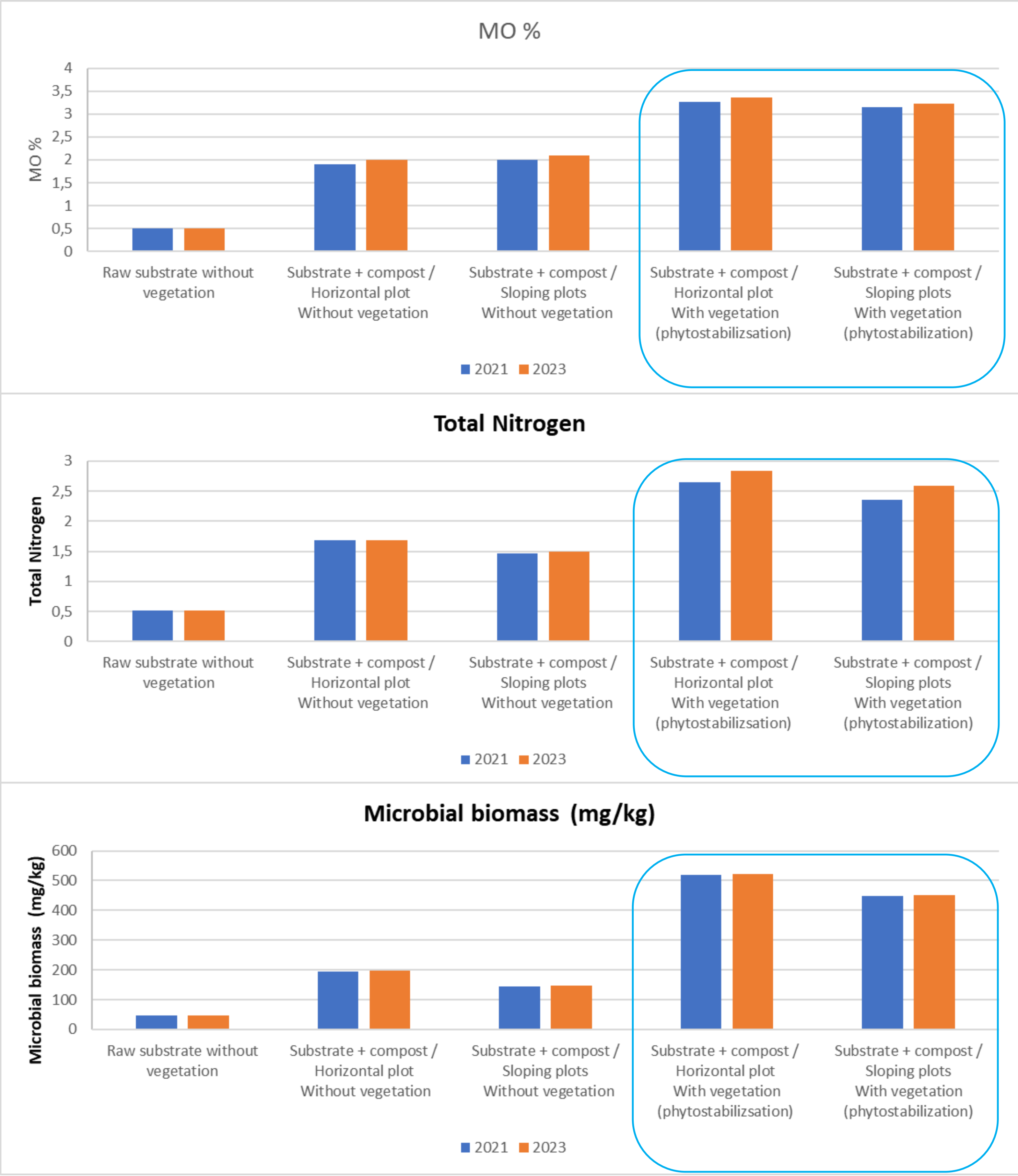


Figure NS2.15**:** Levels of organic matter, nitrogen content and microbial biomass in the substrate of the test plots (changes between 2021 and 2022)

Biomass of aerial parts

Five samples of aerial parts were taken for each sub-plot. The leaves and stems inside the cylinder (0.08 m^2) were harvested (Figure NS2.23).

Figure NS2.**16:** Cylinder used for harvesting aerial parts

The plants were then dried at 60 ° C for 1 week and the dry biomass of each sampling point was measured.

The monitoring of changes in plant cover at the level of the test plots shows very good coverage for the sub-plots that were sown with metal-tolerant species (Figures NS2. 24 and 25). The biomass of the aerial parts is very satisfactory (Figure NS2.26).

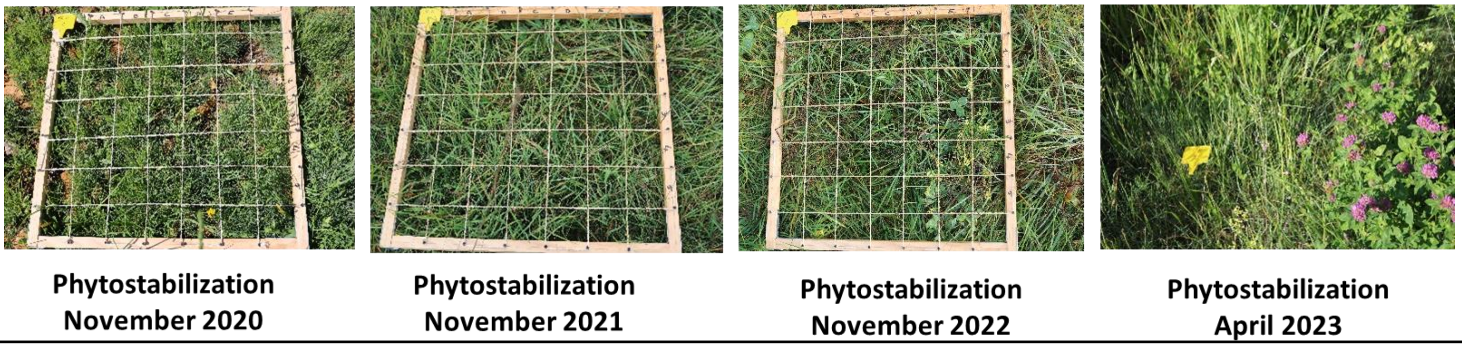


Figure NS2.17: Changes in plant cover at the level of the test plots

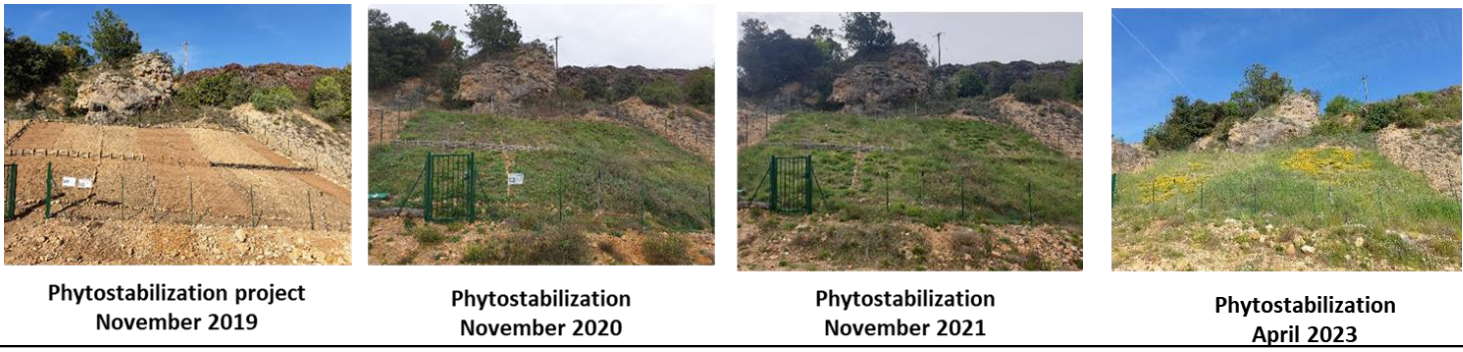


Figure NS2.18: Changes in the vegetation cover at the sloping test plots

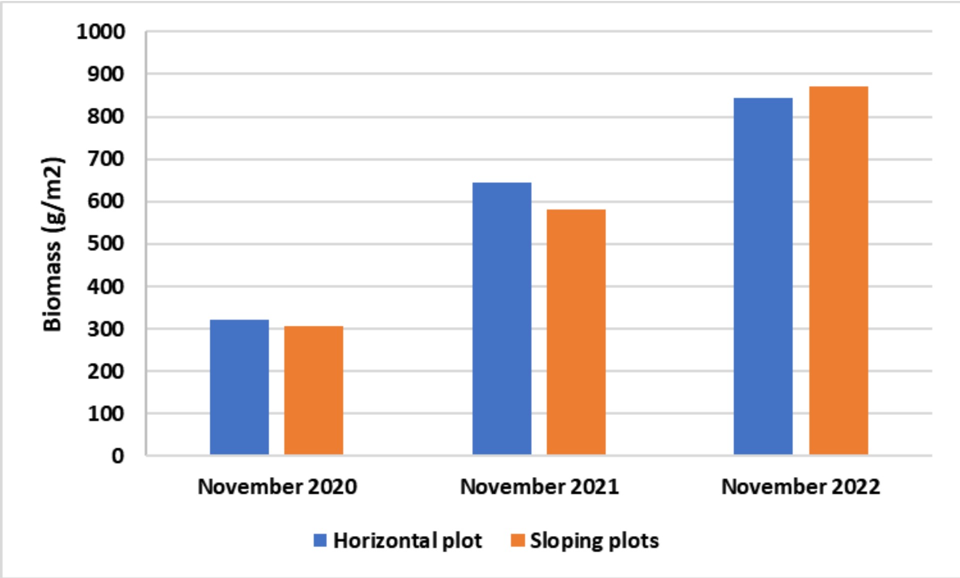


Figure NS2.19: Changes in DW yield of the vegetation cover in the test plots

In addition, more than 3 years after sowing, the sown species show satisfactory fluorescence (Figure NS2.27) and aerial biomass (Figure NS2.28).

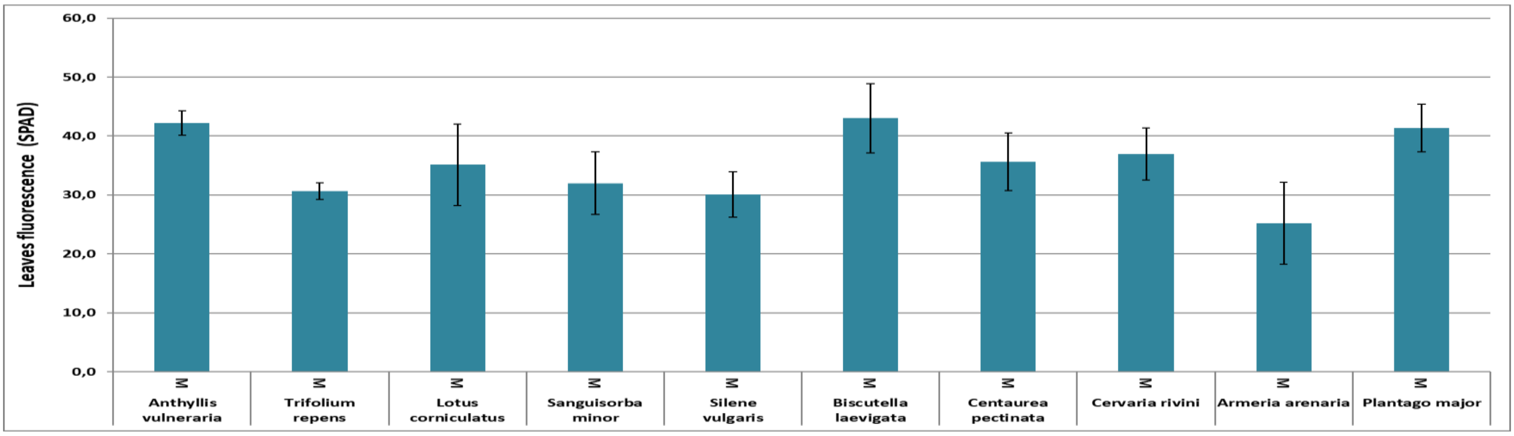


Figure NS2.20: Fluorescence of leaves (SPAD)

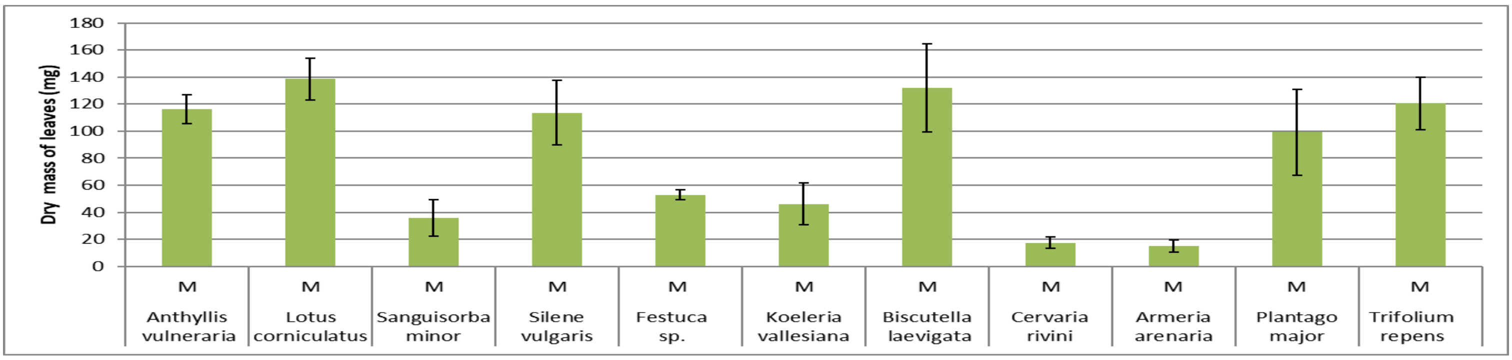


Figure NS2.21: biomass of aerial parts of all species sown and found in test plots

➢ More than 3 years after sowing, the sown species show a non-hyperaccumulation of Zn, Cd, Pb and Tl in aerial parts (Figure NS2.29).

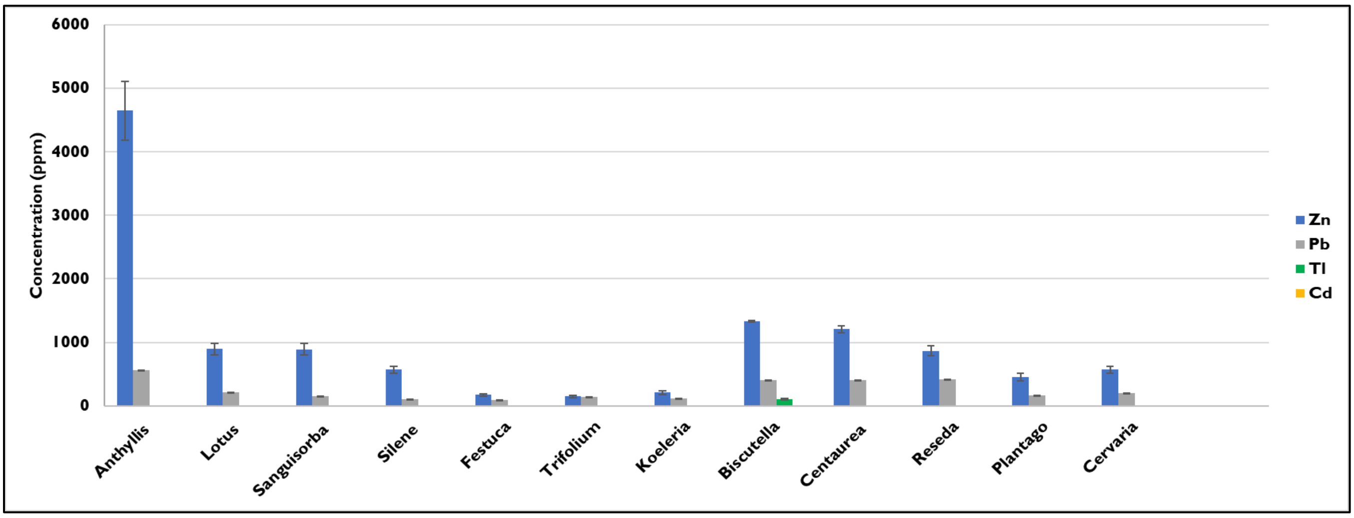


Figure NS2.22: Metal content in harvested aerial parts

Mycorrhization rate analysis

Root system samples were taken in late spring 2021. The roots were washed and stained for assessing the mycorrhization rate. Samples were taken from different plots for comparisons.

➢ The sloping plots have values similar to the vegetation naturally present on the site for all the indices. On the other hand, the frequency of mycorrhization (Figure NS2.30) and arbuscular intensity (Figure NS2.31) are higher at the level of the roots of plants in the Horizontal plot.

➢ Determination of mycorrhization rates 3 years after sowing shows a slight increase in mycorrhization frequency (Figure NS2.30) and arbuscular intensity (Figure NS2.31) for all the zones observed.

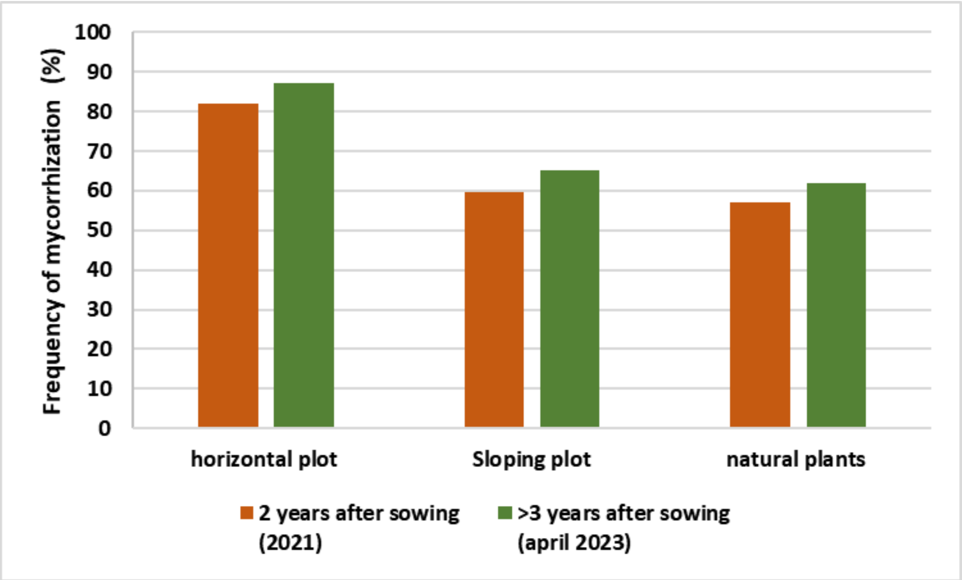


Figure NS2.**23:** Comparison of the mycorrhization frequency between the plants of the horizontal plot, the sloping plots and the plants naturally present on site (data in 2021 and 2023)

Figure NS2.**24:** Comparison of the arbuscular intensity between the plants of the horizontal plot, the sloping plots and the plants naturally present on site (data from 2021 and 2023)

Analysis of fungal diversity

The aim was to collect soil samples from all over the site and from a variety of conditions, so as to be able to compare between barren, naturally vegetated, and phytostabilized soils, with different configurations (vertical/horizontal). All samples (Table NS2.6) were processed to assess the diversity of soil fungal communities.

Table NS2.**5:** Summary of samples taken.

Phyto: Phytostabilization; P.: Plant; T0: Substrate installation; Veg: Vegetation

Samples	Localisation
SLLM1	Résidus nus Phyto 2012
SLLM2	Résidus Veg Phyto 2012
SLLM3	Résidus nus phyto 2002
SLLM4	Résidus veg Phyto 2002
SLLM5	Stériles nus en pente
SLLM6	Stériles nus Horizontale
SLLM7	P. Horizontale veg dense
SLLM8	P. Horizontale veg très dense
SLLM9	T0 : P. Horizontale Substrat + Compost
SLLM10	T0 : P. Pente Substrat + Compost
SLLM11	P. Pente veg dense
SLLM12	P. pente veg - dense

Assessment of soil fungal community diversity

Analysis design

The analysis aimed at assessing the diversity of soil fungal communities in various environments on the Avinières site, and to compare these samples with each other for determining whether or not fungal communities differed according to location, strategy or changes over time.

Analysis of fungal diversity using Illumina sequencing

After collecting soil samples on site, DNA extraction was performed on fresh soil samples using a soil-specific extraction kit (FastDNATM Kit for Soil, MP Biomedicals, Santa Ana, CA). Specific amplifications of the target DNA fragment (ITS2) by PCR were performed on all samples. Sequencing was performed on a 2x300bp Illumina MiSeq sequencer using v3 chemistry. Raw data processing (cleaning, sorting, etc.) was carried out before result analysis (taxonomic assignment, index calculation, comparisons, and graphs) with R software.

Illumina soil sequencing results

Raw sequencing data and processing

In all, over 460,000 sequences were generated for all samples. Table NS2.7 shows the number of sequences obtained for each sample and the number of fungal sequences identified (127413 in total) from the total number of sequences. After sorting and cleaning the raw data, taxonomic assignment was carried out using a customized version of the ITS UNITE v7 database. Sequences without primers, sequences smaller than 32 nucleotides and sequences with uncalled ("N") bases were eliminated. To create the final table of identified fungal species, additional filters were applied to retain only good-quality sequences.
From the final sequence table, diversity indices and fungal community composition comparisons were performed on each group. A data rarefaction step, i.e. a reduction of each sample to the same number of sequences, was carried out. This step was carried out by group or not, in order to retain as much information as possible and to compensate for differences in the number of sequences between samples.
It should be noted that the sequencing of sample SLLM11 did not function correctly, resulting in a low number of sequences being generated. This sample could not be included in the bioinformatics analyses and comparisons with the other samples, as it is too different from the others.

Table NS2.**6**: Number of sequences generated by Illumina sequencing

Phyto: Phytostabilization; P.: Plank; T0: Substrate installation; Veg: Vegetation

Samples	Localisation	Reads	Fungal sequences
SLLM1	Résidus nus Phyto 2012	41479	1082
SLLM2	Résidus Veg Phyto 2012	35408	10272
SLLM3	Résidus nus phyto 2002	47971	9714
SLLM4	Résidus veg Phyto 2002	38838	15649
SLLM5	Stériles nus en pente	73229	13432
SLLM6	Stériles nus Horizontale	65128	19605
SLLM7	P. Horizontale veg dense	39096	14533
SLLM8	P. Horizontale veg très dense	40972	16735
SLLM9	T0 : P. Horizontale Substrat + Compost	4228	1028
SLLM10	T0 : P. Pente Substrat + Compost	37355	8663
SLLM11	P. Pente veg dense	106	31
SLLM12	P. pente veg - dense	43724	16669

Based on the sequences identified as fungal sequences, the following analyses were carried out:

- Calculation of diversity indices

- Fungal community composition (different Phylum)
- Comparison of fungal guilds (fungal category)

The number of fungal species observed ranged from 31 to 109, depending on the type of sample (Table NS2.8). For vegetated tailings (SLLM2 and SLLM4), the number of species is higher than for bare tailings (SLLM1 and SLLM3). The same observation is made for the comparison between sample SLLM5 (bare tailings with 32 species) and different vegetated locations (SLLM 2-4-8-12) where samples present between 76 and 104 species. This is also confirmed in different configurations such as sloping (SLLM5 // SLLM 10-12) and horizontal (SLLM6-9 // SLLM7-8).
In terms of other indices of species diversity and distribution, all the vegetated samples showed a high level of diversity and a good distribution of species compared with most of the bare soil samples. Bare soil sample SLLM1, on the other hand, showed similar values to the vegetated sample (SLLM2), with a lower number of species (SObs). Sample SLLM5 (bare soil) also shows high indices, but the number of species is one of the lowest (Table NS2.8).

Table NS2.**7:** Total fungal community diversity indices for each sample.

Phyto: Phytostabilization; P.: Plank; T0: Substrate placement; Veg: Vegetation

Samples	Localisation	Sobs	Shannon	Inv_Simpsor	Pielou
SLLM_1	Résidus nus Phyto 2012	64	3,41	18,69	0,82
SLLM_2	Résidus Veg Phyto 2012	104	3,31	11,44	0,71
SLLM_3	Résidus nus phyto 2002	31	2,06	3,63	0,60
SLLM_4	Résidus veg Phyto 2002	76	2,54	4,77	0,59
SLLM_5	Stériles nus en pente	32	2,82	11,19	0,81
SLLM_6	Stériles nus Horizontale	70	2,79	8,00	0,66
SLLM_7	P. Horizontale veg dense	109	3,48	17,64	0,74
SLLM_8	P. Horizontale veg très dense	98	3,56	17,30	0,78
SLLM_9	T0 : P. Horizontale Substrat + Compost	57	1,93	2,87	0,48
SLLM_10	T0 : P. Pente Substrat + Compost	37	1,73	2,58	0,48
SLLM_12	P. pente veg - dense	88	2,94	7,92	0,66

- ➢ The analysis of diversity indices shows that when a soil in different configurations is vegetated, the number and diversity of fungal species is greater than when the soil is bare.

Analysis and comparison of fungal diversity

In order to gain a better understanding of the differences in the taxonomic composition of the different samples, several comparisons were made. The following results are presented by group according to the characteristics to be compared.

➢ **Bare and vegetated residues**

Samples showed a similar total fungal community composition, with a dominance of one major Phylum, the Ascomycota (Figure NS2.32). Compositions varied little between barren and vegetated soils. Nevertheless, the Basidiomycota Phylum was more abundant in the 2012 SLLM1 bare soil than in the SLLM2 vegetated soil.
Among the diversity of fungal communities, Glomeromycota (mycorrhizal fungi associated with roots) were found in low quantities in all samples. Basidiomycota, which were the most abundant in sample SLLM1, was the most diverse in this sample (Figure NS2.33). In the other three samples, this Phylum was represented by an equivalent number of Divisions. As for Ascomycota (the most abundant Phylum in all samples), diversity was equivalent for all four samples, with different Division compositions for each sample. On the other hand, the Rozellomycota Phylum was a marker of vegetated soils, as this Division was specific to the two samples SLLM2 and SLLM4 (Figure NS2.33).

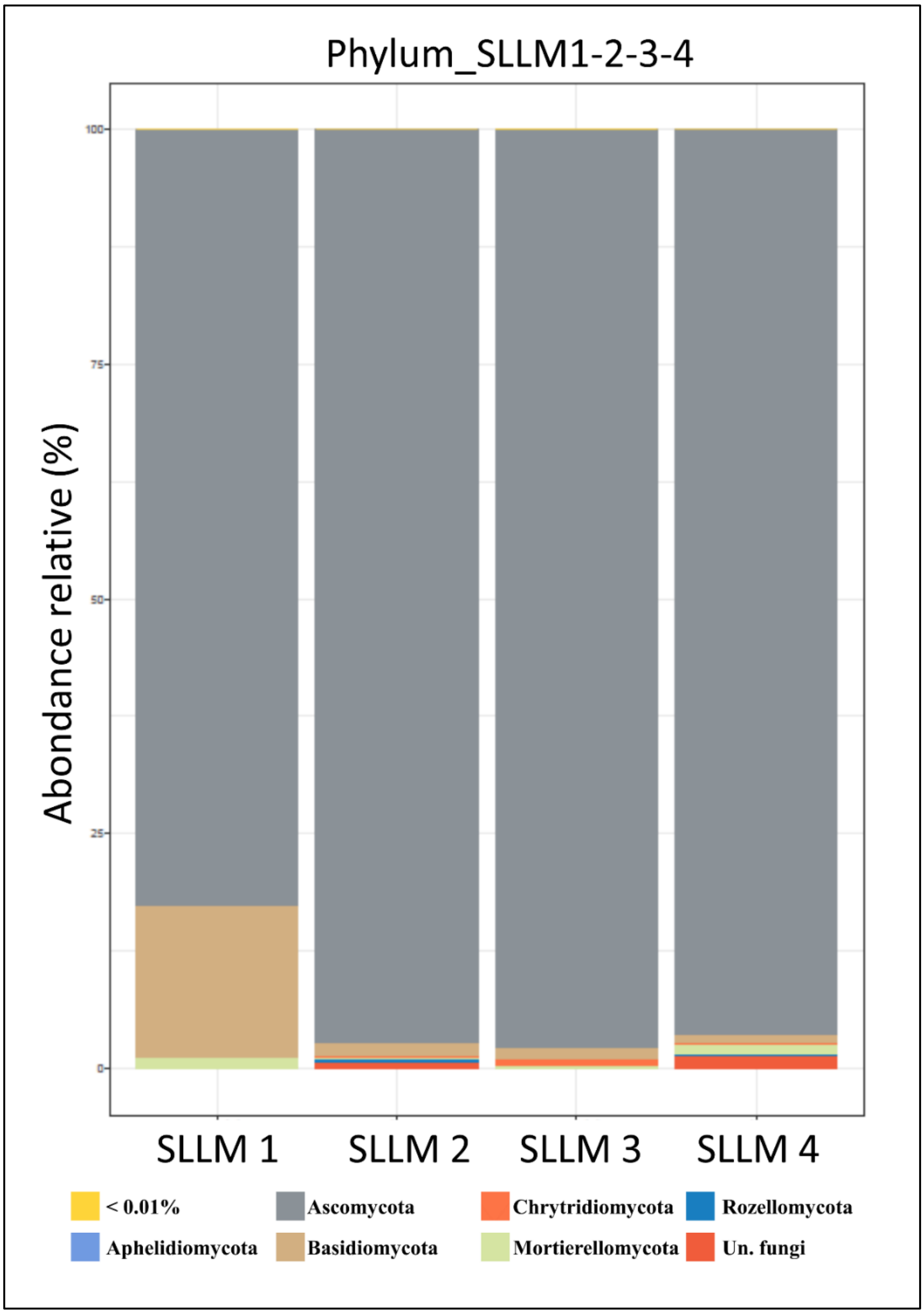


Figure NS2.25: Comparison of soil fungal community composition, at the Phylum taxonomic level, for the four bare and vegetated soil samples at residue level. Each color represents a phylum of fungi

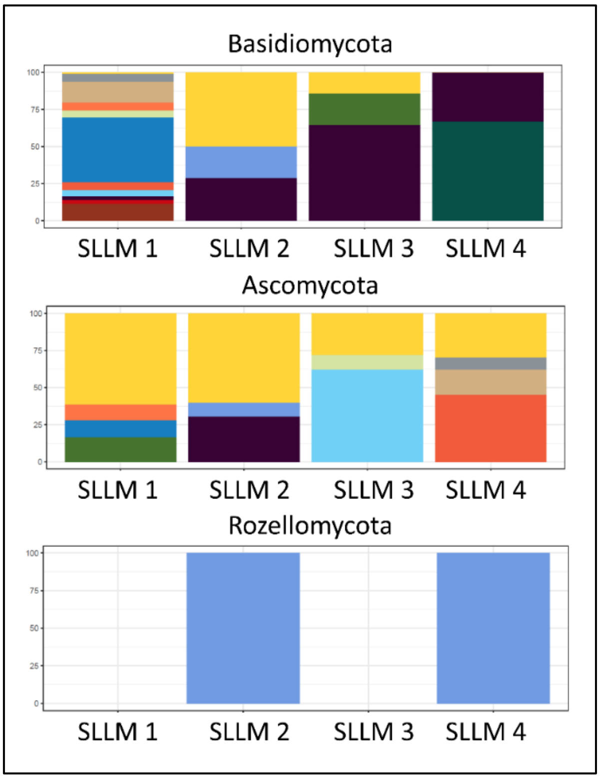


Figure NS2.26: Comparison of the composition of certain Phyla for the four bare and vegetated soil samples at residue level. Each color represents a branch of a Division of the Phylum concerned.

Regarding the fungal guilds (fungal function) that make up the observed communities, the soil compositions were virtually similar between the four samples. All samples showed a wide variety of guilds, with differences in the abundance of certain guilds between bare and vegetated soils (Figure NS2.34). Both bare soils (SLLM1 and SLLM3) showed a strong presence of animal pathogenic fungi. Conversely, wood-saprotrophic fungi increased sharply in both vegetated soils (SLLM3 and SLLM4), and these latter were the only ones to show lichenized fungi (Figure NS2.34).

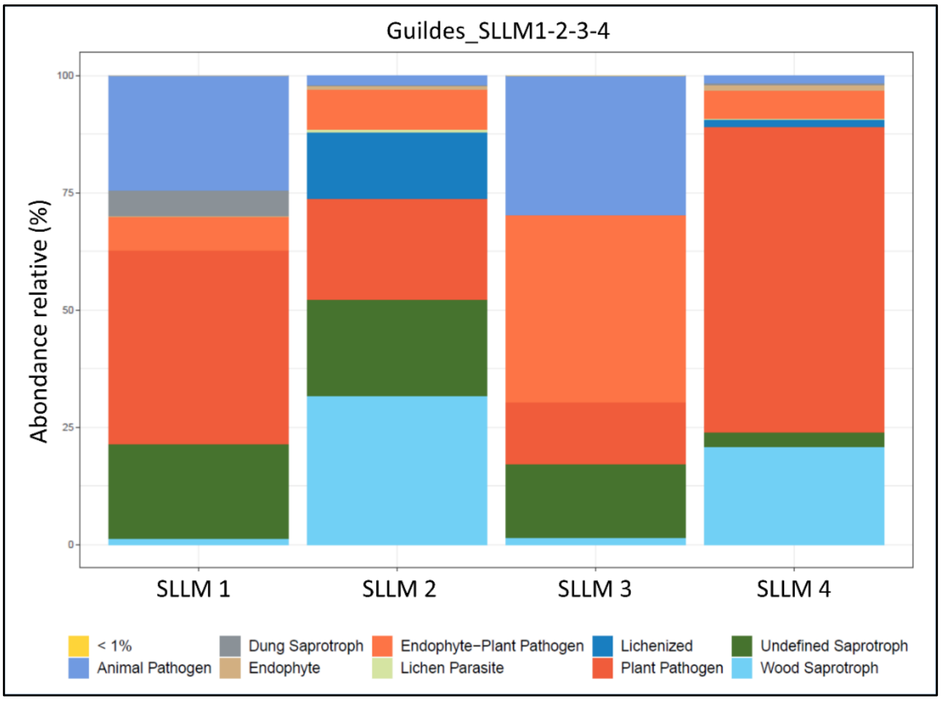


Figure NS2.27: Comparison of the composition of the different fungal guilds in the soil community for the four bare and vegetated soil samples at residue level. Each color represents a fungal guild.

In terms of specificity, both vegetated soils (SLLM2 and SLLM4) showed an increase in the number of species (Sobs), but with roughly identical diversity (Table 8).

➢ The different samples showed a fungal diversity that was more or less identical between bare and vegetated soils. Different fungal groups emerged with revegetation, and certain fungal groups were able to serve as markers of soil type (bare / revegetated).

➢ **Different vegetated soils**

This comparison was designed to compare different types of vegetated soil (SLLM1-4-8-12) with bare soil (SLLM5).

The samples showed a similar total fungal community composition, with dominance by one major Phylum, the Ascomycota (Figure NS2.35). Compositions varied little between vegetated soils (SLLM8-12-2-4) but differed from bare soil (SLLM5). The latter had a strongly represented Phylum, the Chytridiomycota, some genera of which were root parasites. This Phylum decreased sharply with the presence of vegetation. Moreover, the Basidiomycota phylum was more abundant in vegetated soils.
Among the diversity of fungal communities, Glomeromycota (mycorrhizal fungi associated with roots) were found in low quantities in all samples. Basidiomycota, which were the most abundant in vegetated samples, were the most diverse in these samples (Figure NS2.36). The composition of this Phylum differed between the vegetated samples from the residues (SLLM2-4) and those from the horizontal and sloping beds. Both densely vegetated samples shared a common Coprinopsis group.
As for the Ascomycota (the most abundant phylum in all samples), diversity was similar for all samples, with different compositions in Division for each sample. On the other hand, the Mortierellomycota Phylum was as a potential marker of vegetated soils, as this Division was specific to three samples SLLM8, SLLM2 and SLLM4 (Figure NS2.36).

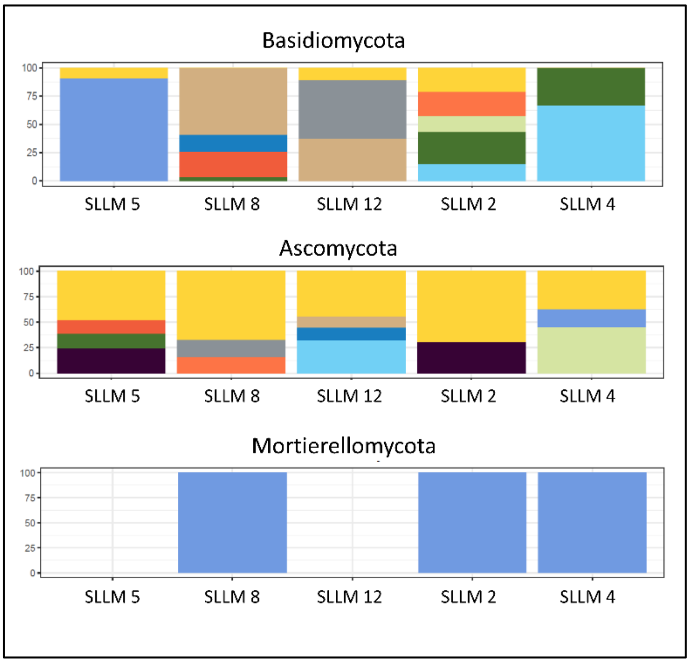


Figure NS2.28: Comparison of selected Phylum compositions for the five vegetated soil samples (SLLM8-12-2-4) compared with bare soil (SLLM5). Each color represents a branch of a Division of the Phylum concerned.

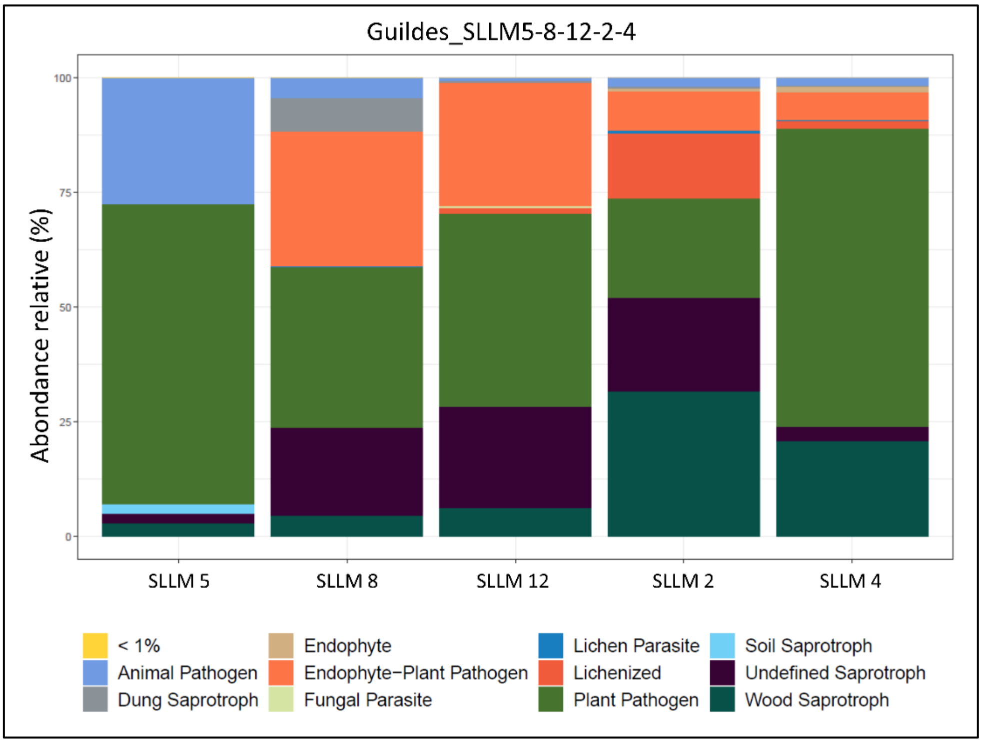


Figure NS2.29: Comparison of the composition of different fungal guilds in the soil community for the five vegetated soil samples (SLLM8-12-2-4) compared with bare soil (SLLM5). Each color represents a fungal guild.

- The fungal diversity of the different samples was similar between bare and vegetated soils. Different fungal groups emerged with revegetation, and certain fungal groups would serve as markers of soil type (bare/vegetated).

- **<u>Different sloping soils</u>**

This comparison was designed to compare a bare soil with two soils amended and not amended with compost.

The fungal diversity of the different samples was virtually identical between bare soil and compost-amended and non-vegetated soil. Different fungal groups emerged with substrate modification, and certain fungal groups would serve as markers of soil type (bare / rehabilitated) (Figures NS2. 37&38).

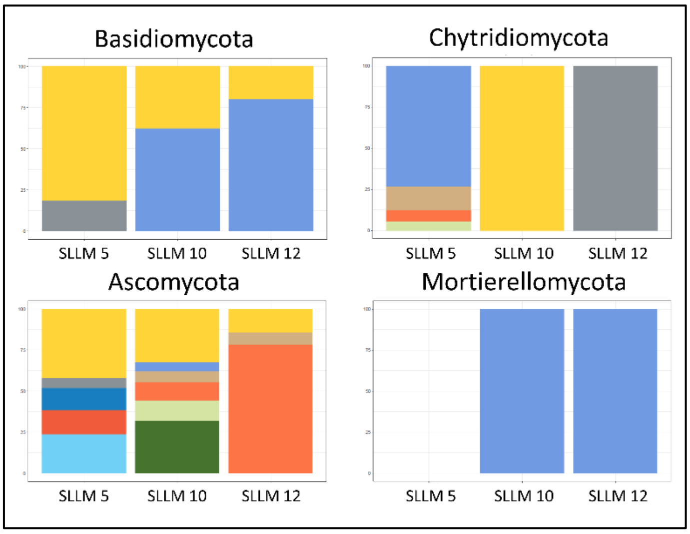


Figure NS2.30: Comparison of the composition of certain Phyla, for the three soil samples on vegetated (SLLM12) and bare (SLLM5-10) slopes. Each color represents a branch of a Division of the Phylum concerned.

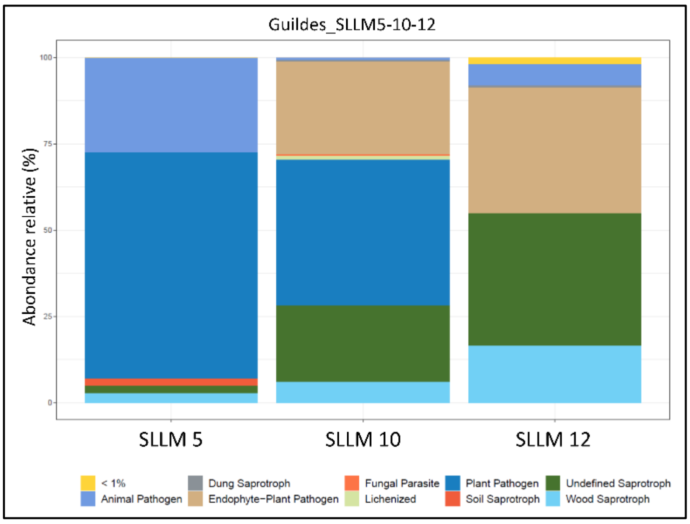


Figure NS2.31: Comparison of the composition of the different fungal guilds in the soil community for the three soil samples on vegetated (SLLM12) and bare (SLLM5-10) slopes. Each color represents a fungal guild.

➢ **Different horizontal soils**

This comparison compares bare soil with two vegetated soils and the non-vegetated compost-amended soil.

The fungal diversity of the different samples was similar between bare soil and compost-amended and non-vegetated soil. Different fungal groups emerged with substrate modification, and certain fungal groups would serve as markers of soil type (bare / rehabilitated) (Figures NS2.39-41).

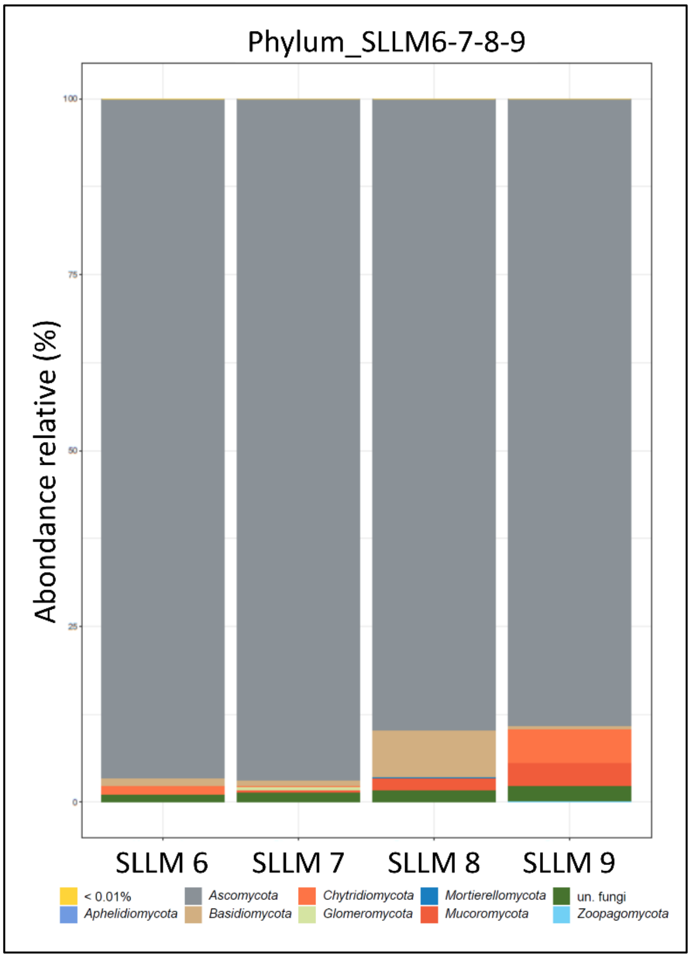


Figure NS2.32: Comparison of soil fungal community composition, at Phylum taxonomic level, for the four soil samples in vegetated (SLLM7-8) and bare (SLLM6-9) horizontal beds. Each color represents a phylum of fungi.

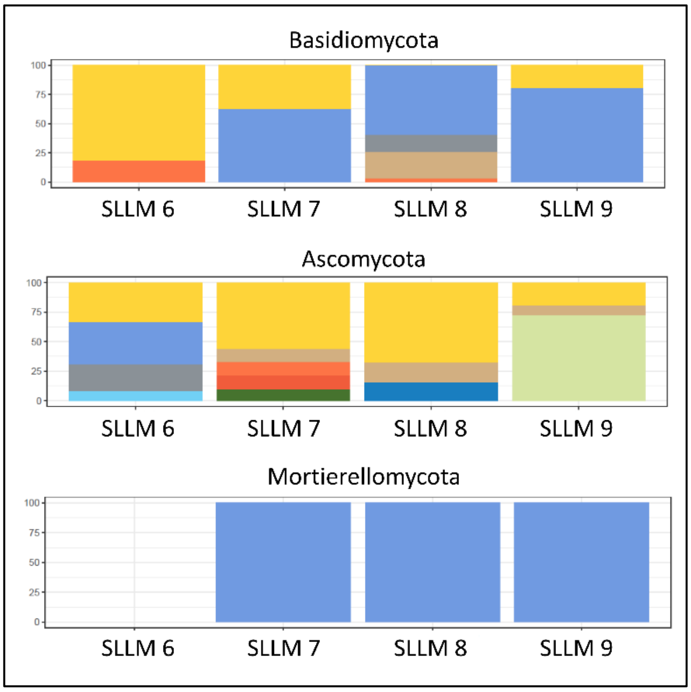


Figure NS2.33: Comparison of the composition of certain Phyla, for the four soil samples in horizontal vegetated (SLLM7-8) and bare (SLLM6-9) beds. Each color represents a branch of a Division of the Phylum concerned.

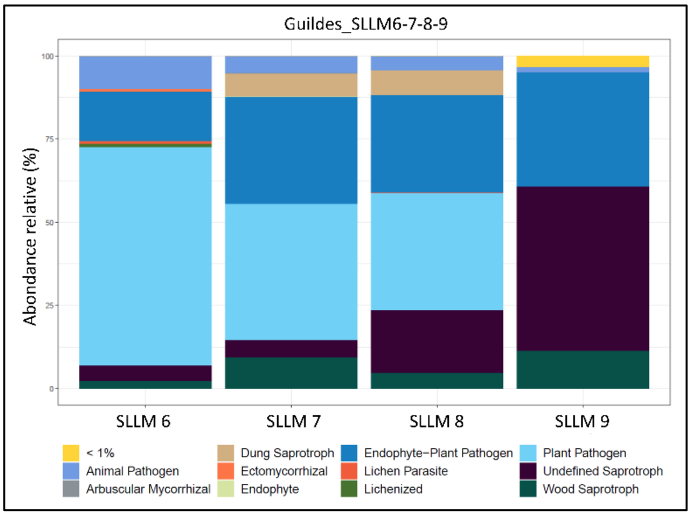


Figure NS2.34: Comparison of the composition of the different fungal guilds in the soil community for the four soil samples in horizontal vegetated (SLLM7-8) and bare (SLLM6-9) beds. Each color represents a fungal guild.

Conclusion on the diversity of soil fungal community

For all the data, it was difficult to find general principles for all the samples, given the diversity of environments on the site (slope, type of residue). Nevertheless, globally, vegetated soils displayed greater diversity in the fungal community and a higher number of species. Furthermore, mine tailings showed greater diversity than pond tailings.

The composition of the fungal community varied between the samples, and certain Phyla would be markers between vegetated and non-vegetated soils. Bare soils showed a strong presence of Chytridiomycota (potential root parasites), which decreased with the presence of vegetation. Then, modified soils (composting) and vegetated soils (densely or not) displayed a strong presence of several Phylum such as Mortierellomycota (root-associated fungi), Colletotrichum and Humicola.

Certain fungal functions also reflected either bare or vegetated soil. Saprotrophic fungi dominated in vegetated soils, whereas bare soils were dominated by fungal pathogens of animals and plants.

Certain fungi groups would be potential markers of either vegetated or bare soils, and it would be interesting to compare these data with samples from other mine sites to confirm these observations.

Phytostabilizing slopes with Dorycnium sp

Sowing of Dorycnium

Dorycnium seeds (wild seeds collected on site) were sown in terrines under controlled conditions on September 2021. After germination (Figure 42) and root system development (Figure NS2.43), the seedlings were transplanted into cups (Figure 43). A total of 1 700 seedlings were transplanted**.**

Figure NS2.35: Germination and growth of Dorycnium seedlings in terrines

Figure NS2.36: Root system development of Dorycnium seedlings

Figure NS2.37: *Dorycnium* seedlings in culture after transplanting

Dorycnium sp. pilosa cuttings

Sampling of Dorycnium at the Avinières site was carried out on October 2021 (Figure NS2.45). Cutting began in the nursery on October 06. A total of 1 000 Dorycnium cuttings were prepared and planted (Figures NS2.46).



(1) **(2)** **(3)**

(1) Figure NS2.38: Sampling of *Dorycnium* twigs at the Avinières site**. (2) Figure NS2.39:** *Dorycnium* cuttings in the nursery. (3) **Figure 40:** Planting *Dorycnium* cuttings in the nursery

Bio-fertilization

Each species was bio-fertilized (Figure NS2.48).

Figure NS2.41: Bio-fertilization of *Dorycnium* plants grown in nurseries

The plants were raised in the nursery for 1 year. At the end of this phase, the plants were delivered and planted on site. A total of 1 880 plants were grown for 1 year in the nursery and then planted at Les Avinières.

Figure NS2.42: Planting of metallicolous shrubs September 2022

The recovery rate was evaluated in April 2023: a satisfactory recovery rate was observed: 90% of the plants recovered and flowering was observed on several plants (Figure NS2.50).

Figure NS2.43: Flowering of *Dorycnium* plants planted in autumn 2022

Degradation of organic matter

In order to monitor the degradation of organic matter naturally supplied by plants installed after phytostabilization, LEVA-bag® were placed at a depth of around 15 cm at several locations on the test plots at Les Avinières site. Control areas were also involved for comparison.

The LEVA-bag® is a tool used to measure the degradation of a reference organic material (straw) in a small nylon bag. The LEVA-bag measured the rate of degradation of crop residues by calculating the loss in mass over time resulting from the activity of decomposer organisms passing through the mesh and micro-organisms.

Some of the LEVA-bags were collected for analysis 6 months after installation (March 2023). The second part will be harvested in September 2023 to monitor changes in organic matter degradation.

6 months after implementing the LEVA-bags, the vegetated soils displayed lost mass and were close to the unpolluted control.

Regarding the non-vegetated soil, a slight loss in mass was evidenced, showing the presence of microorganisms, but in small quantities (Figure NS2.51).

- Phytostabilization of the Avinières with biofertilization and the addition of compost amendments had enabled us to recreate a functional environment with satisfactory organic matter degradation (close to that of unpolluted soil). Further monitoring will be carried out in autumn 2023 to follow changes in organic matter degradation.

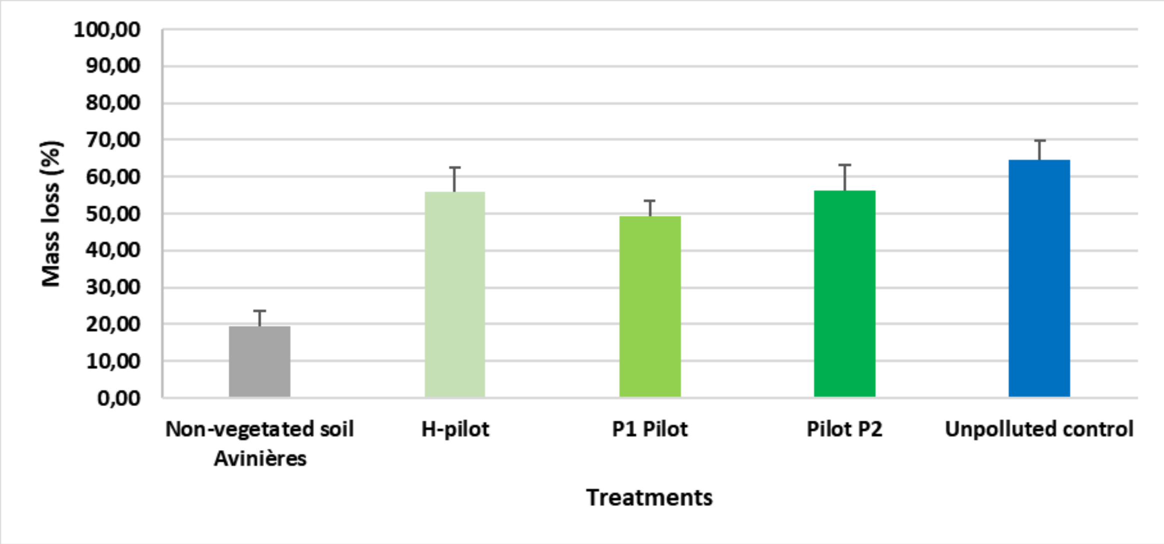


Figure NS2.44: Average mass loss of Leva-bags for the different treatments for the March 2023 harvest.

H-Pilot: horizontal trials; P1-Pilot: Pilot 1 on slope; P2-Pilot: Pilot 2 on slope

References

Bailly-Maitre MC. 1990. Pour une histoire des mines au Moyen-âge, l'exemple des mines de la Terre d'Hierle (Gard). *Archéologie Du Midi Médiéval* **VII**: 61–71.

Balabane, M., Faivre, D., van Oort, F., Dahmani-Muller, H., 1999. Mutual effects of organic matter dynamics and heavy metal fate in a metallophyte grassland. *Environmental Pollution*, **105**, 45-54.

Bouladon, J., 1977. Les gisements de plomb-zinc-argent du Massif Central. *Bulletin du B.R.G.M.*, Section II, 67-90.

Dahmani-Muller, H., van Oort, F., Gélie, B., Balabane, M., 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution*, **109**, 231-238.

Escarré, J., Lefebvre, C., Gruber, W., Leblanc, M., Lepart, J., Riviere, Y., Delay, B., 2000. Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New Phytologist,* **145**, 429-437.

Escarré J., Lefèbvre C., Raboyeau S., Dossantos A., Gruber W., Cleyet Marel JC., Frérot H., Noret N., Mahieu S., Collin C., van Oort F., 2011. Heavy metal concentration survey in soils and plants of the Les Malines mining district (Southern France): Implications for soil restoration. *Water, Air, & Soil Pollution*, **216,** 485–504.

Frérot, H., Lefèbvre, C., Gruber, W., Collin, C., Dos Santos, A., Escarré, J., 2006. Specific interactions between local metallicolous plants improve the phytostabilization of mine soils. *Plant and Soil,* **282**, 53–65.

Mahieu, S., Escarré, J., Brunel, B., Méjamolle, A., Soussou, S., Galiana, A., Cleyet-Marel, JC., 2014. Soil nitrogen balance resulting from N fixation and rhizodeposition by the symbiotic association *Anthyllis vulneraria/Mesorhizobium metallidurans* grown in highly polluted Zn, Pb and Cd mine tailings. *Plant and Soil,* **375**, 175-188

Mahieu, S., Frérot, H., Vidal, C., Galiana, A., Heulin, K., Maure, L., Brunel, B., Lefèbvre, C., Escarré, J., Cleyet-Marel, JC., 2011. *Anthyllis vulneraria/Mesorhizobium metallidurans*, an efficient symbiotic nitrogen fixing association able to grow in mine tailings highly contaminated by Zn, Pb and Cd. *Plant and Soil,* **342**, 405-417.

Mahieu, S., Soussou, S., Cleyet-Marel, JC., Brunel, B., Maure, L., Lefebvre, C., Escarré, J., 2013. Local adaptation of metallicolous and non-metallicolous *Anthyllis vulneraria* populations: Their utilization in soil restoration. *Restoration Ecology*, **21**, 551-559.

Néel, C., Bril, H., Courtin-Nomade, A., Dutreuil, J.P., 2003. Factors affecting natural development of soil on 35-yeard-old sulphide-rich mine tailings. *Geoderma*, **111**, 1-20.

Rolley JP., 2002. La petite histoire du Plomb et du Zinc en Cévennes. [document WWW] URL http://www.ensm-ales.fr/ ~jprolley/Geologie/ Pb-Zn.html.

Soussou, S., Mahieu, S., Brunel, B., Escarré, J., Lebrun, M., Banni, M., Boussetta, H., Cleyet-Marel, JC., 2013. Zinc accumulation patterns in four *Anthyllis vulneraria* subspecies supplemented with mineral nitrogen or grown in the presence of their symbiotic bacteria. *Plant and Soil,* **371**, 423-434

Van Haluwyn, C., Petit, D., Mériaux, J.-L., 1987. Végétations métallicoles dans la région Nord - Pas-de-Calais. *Bulletin de la Société Botanique Nationale de France*, **40**, 7-15.

van Oort, F., Balabane, M., Dahmani-Muller, H., Jongmans, A.G., Nahmani, J., 2002. Approches intégrées du fonctionnement d'une pelouse métallicole. In Baize D., Tercé M., Eds. *Les Eléments métalliques dans les sols - Approches fonctionnelles et spatiales*. INRA-Éditions, Paris, 313-330

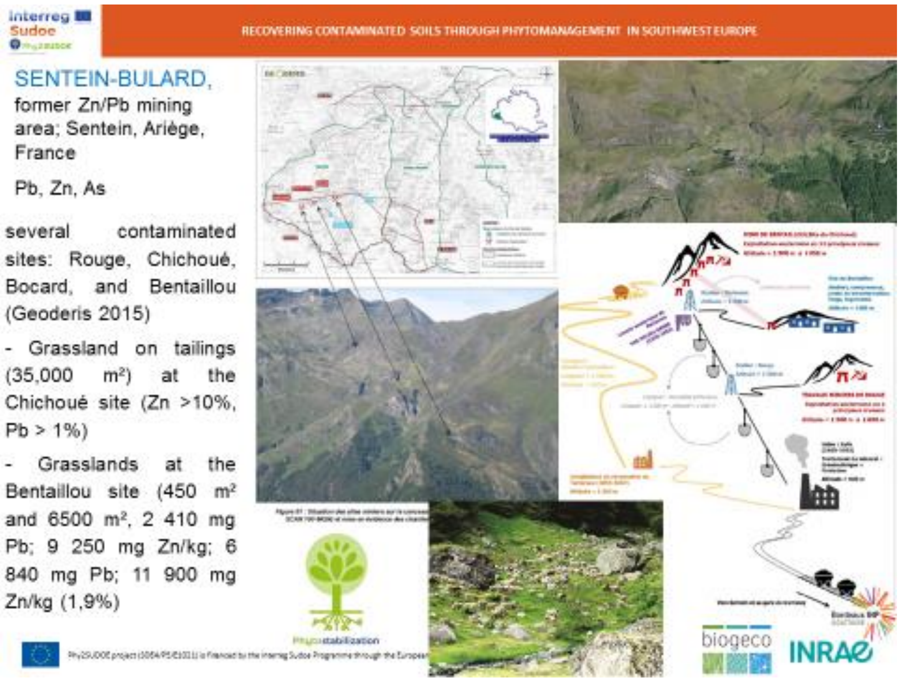
van Oort, F., Labanowski, J., Jongmans, A.G., Thiry, M., 2008. Devenir des polluants métalliques dans les sols: révélateur d'impacts de l'activité humaine sur la pédogenèse. *Etude et Gestion des Sols*, numéro en hommage à Michel Robert,

van Oort, F., Thiry, M., Jongmans, T., Bourennane, H., Cambier, P., Lamy, I., Citeau, L., Fernandez, C., Nahmani, J., 2007. Les pollutions métalliques d'un site industriel et des sols environnants : distributions hétérogènes des métaux et relations avec l'usage des sols. *In* Cambier, P., Schvartz, C., van Oort, F., Eds. *Devenir et Effets des Contaminations Métalliques dans les Agrosystèmes et Ecosystèmes Terrestres Péri-industriels*. Editions Quae, Versailles, France

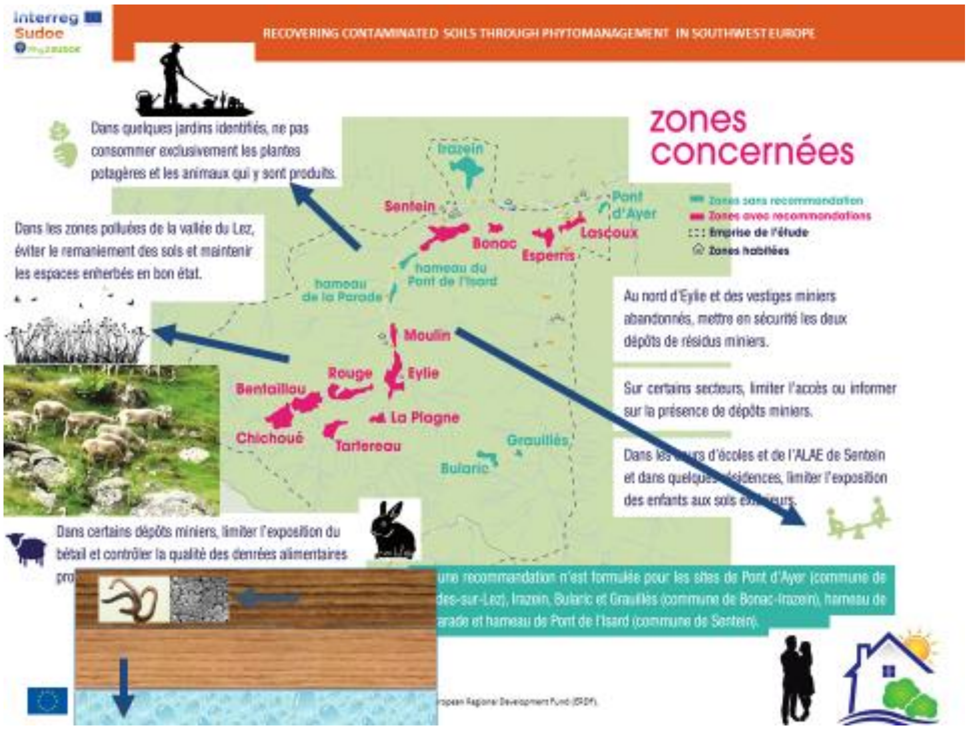
Vidal, C., Chantreuil, C., Berge, O., Maure, L., Escarre, J., Bena, G., Brunel, B., Cleyet-Marel, J.C., 2009. *Mesorhizobium metallidurans* sp. nov., a metal-resistant symbiont of *Anthyllis vulneraria* growing on metallicolous soil in Languedoc, France. International Journal of Systematic and Evolutionary Microbiology, 59, 850-855.

NS3-Sentein:

As part of the inventory of mining wastes from the extractive industry (Article 20 of European Directive 2006/21 / EC, so-called “DDIE” inventory) in France, a survey was carried out on the Bulard, Irazein, Melles, Orle and Sentein concessions (2010 - 2011). At the end, the sector associated with the grouping of these five sites obtained a maximum class (class E). In terms of potential risks, this means that the sector is "likely to present a significant risk to the environment and human health, and that it requires an urgent detailed environmental study, if it has not already been carried out". The specific climate of the area is mountainous with an Atlantic regime. The annual precipitation is in the 1000 - 1500 mm range on average without marked deficit over the whole year, which favors the rain-loving species of the mountain range, e.g. Fir and Beech, and favors forest development up to 1,500 to 1,600 m in general. Monthly precipitation is in the range of 90 to 150 mm. Precipitation in snow form generally begins in October from an altitude of 1,200 m and the average snow cover lasts from November to April. Spring snowfalls can be frequent and abundant and snowy areas can remain in August, above 2,400 m in northern exposed areas. The runoff of pollutants is therefore of concern. The land in the Sentein mining district is developed essentially one sedimentary bedrocks owe their deformation and metamorphism to the Hercynian orogeny (dating from the Carboniferous-Permian). The mineralized zone represents, in vertical projection, a surface of 600 m long and 200 m wide. Lead-zinc ore contains on average 3 times more Zn than Pb, for an overall content of roughly 10% to 15%. Minor metals are Ag, Cd and Ge. The Sentein concession, the most important in this sector, exploited for Zn, Pb and - to a lesser extent - Ag, from 1848 to 1963, for a total production of one million tonnes of ore (grading approximately 10% Zn and 3% Pb, or approximately 125,000 tonnes of metal).



Location of the Sentein plots



Conceptual model for the various contaminated areas in the vicinity of Sentein © INRAE

● **Soil properties**: The topsoils of the toposequence from Le Bocard to Chichoué were analyzed showing a strong relationship between 1M NH_4NO_3-extractable soil Zn (a proxy of potential phytoavailable soil Zn) and soil pH (depending also to total soil Zn due to the former mining/smelting activities). Soils were mainly contaminated by Zn and Pb, but also Cd, Cu and As in a lesser extent.

Sentein (NS3) RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

Sentein Subsite	Clays g/kg	Organic C g/kg	pH	CEC cmol+/kg	Cu mg/kg	1M NH_4NO_3 – ext. Cu µg/kg	Zn mg/kg	Cd mg/kg	As mg/kg	Pb mg/kg
Le Bocard rep 1	34	27,7	7,61	1,48	180	1074	105695	217	123	76295
Le Bocard rep 2		37,1	7,25	1,80	245	1821	184174	431	187	144708
Le Bocard rep 3		30,9	7,47	1,26	194	1026	126173	271	155	93426
Le Bocard rep 4		28,2	7,43	1,30	205	811	128562	264	162	100306
Terril 1	17	45,7	8,5	< 1	169	2497	11749	34	56	5310
La Plagne 1 (Tox+)	48	29,5	6,7	6,79	1108	1212	31030	56	132	6788
La Plagne 2 (Tox=)	65	47,4	6,83	8,10	1152	2945	21656	33	139	8956
La Plagne 3 (Tox-)	95	64,1	5,37	7,53	170	340	4867	8	83	1991
Chichoué 1 (Tox+)	40	28,3	7,81	5,84	322	461	62551	76	1017	44594
Chichoué 2 (Tox=)	42	59,4	7,51	11,42	162	466	23383	47	591	27987
Chichoué 3 (Tox-)	49	50,9	6,03	8,41	67	40	6692	12	353	11220

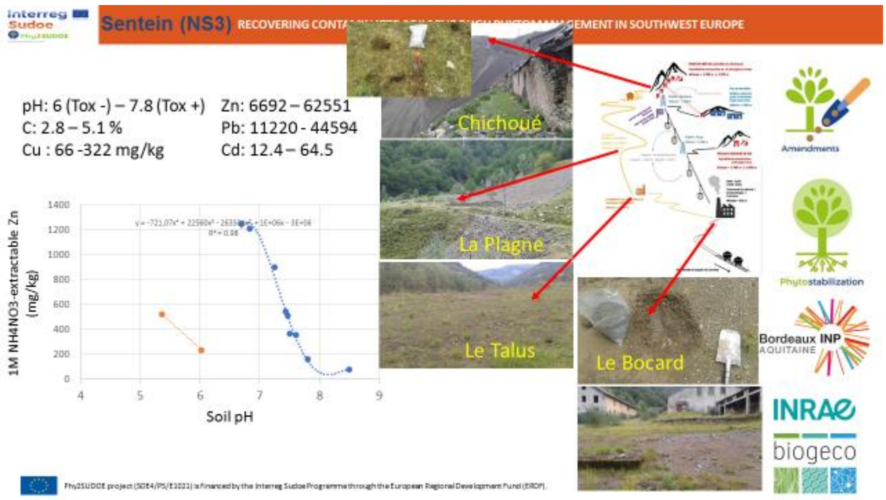
Physico-chemical properties of the Sentein sols © INRAE in coll. Bordeaux INP

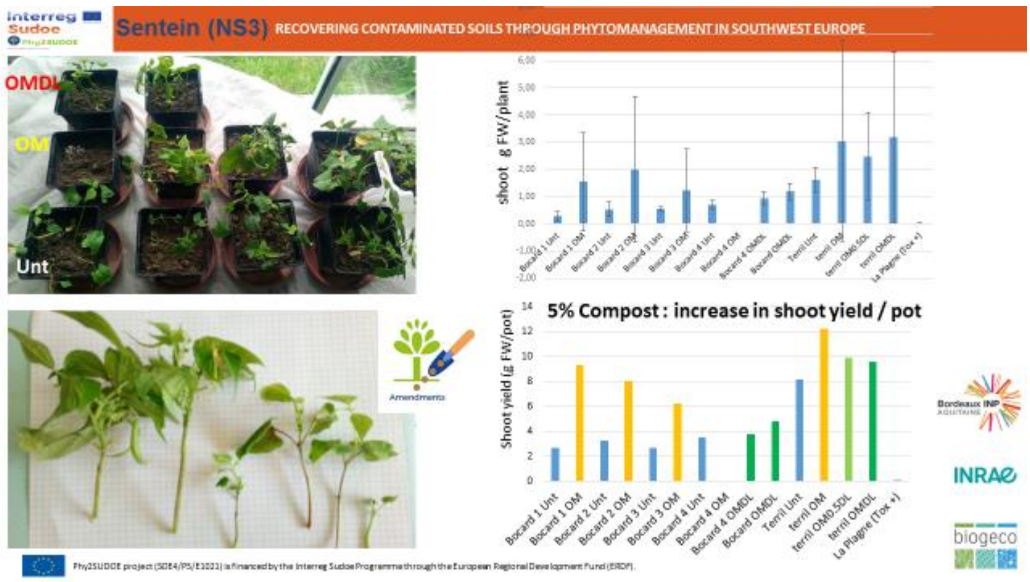
● **Phytomanagement option /plant assembly**

The soil phytotoxicity evidenced in situ is explained by extractable soil Zn (related to soil pH and total soil Zn), and total and extractable soil Cu (in line with organic soil C). Visible symptoms of phytotoxicity were evidenced on shoots in a plant testing with dwarf beans. The incorporation of compost into these soils enhanced the plant growth and decreased the soil phytotoxicity. Seed bank was increased by the compost incorporation. The combination of dolomite with compost was not additionally improving the shoot biomass. Other options combining compost, biochar, bioaugmentation, Cu-tolerant (excluder) grassy species and vermiremediation are investigated since Sept. 2022.

● Metallophyte seeds, plant samples, invertebrates, and microbial strains adapted to increasing contaminant exposures were collected and identified in collaboration with partners and associated partners (Neiker, CSCIC, UPV, UCP-ESB, Fertil'Innov Environnement, Bordeaux INP) and put in collection (Olarizu Germplasm Bank; Biscay Bay Environmental Biospecimen Bank)

● Plant-growth promoting bacteria strains were characterized and used in a pot experiment (UCP-ESB) on soil sample sent by INRAE.





Plant testing on potted sols from the Sentein site: untreated soils (Unt), compost-amended soils (OM), and soils amended with compost and dolomite (OMDL) (© Mench, INRAE)

Tailings (Left) and phytotoxicity testing on the Le Bocard soils, Sentein site © INRAE

Soil sampling for screening microbial community and activity along a contamination gradient at the Sentein site (Chichoué plots) © Mench INRAE / Delerue Bordeaux INP

● Population of Cu-tolerant Agrostis capillaris was assessed to phytostabilize the Sentein soils in combination with compost, biochar and earthworms

Pot experiment with various soil treatments (e.g. compost, biochar and earthworms) to phytomanage the Sentein soils © Mench/INRAE

NS4- Bordes:

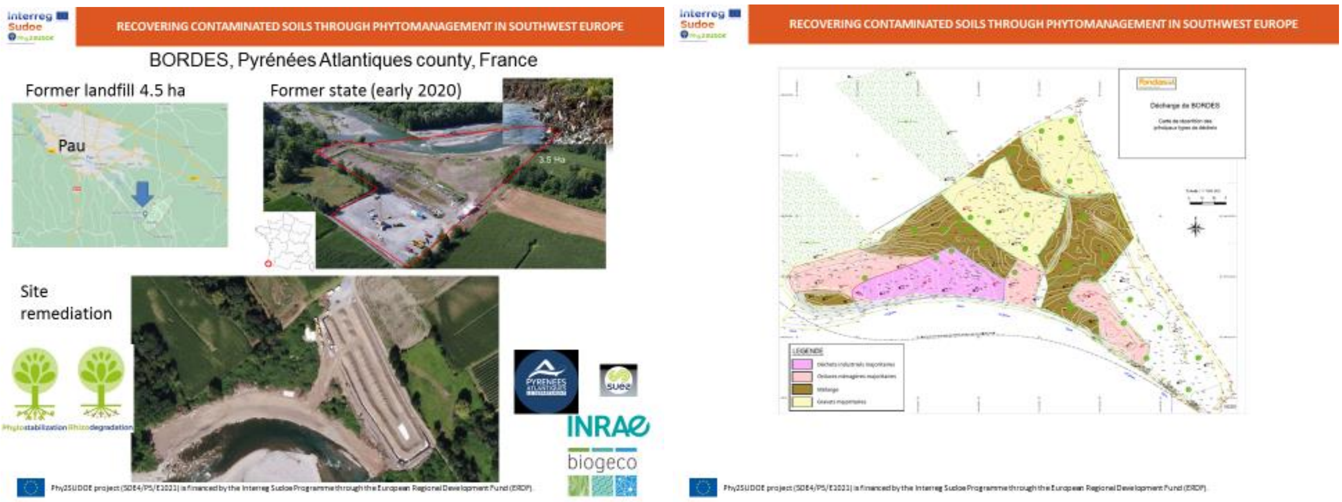
This site is a former landfill supervised by the Bordes town, the community of communes of the Pays de Nay, and the Pyrénées Atlantiques County.

● **Implementation:**

The former landfill of Bordes (64, Pyrénées Atlantiques county, France), in operation from the 1950s until its closure in 1998, is under rehabilitation since 2020 using ecological engineering techniques. The site is progressively returning to nature in the form of a meadow to be reforested over time.

Since the beginning of its operation in 1950, this landfill has received several types of waste (domestic refuse, industrial, rubble, etc.). Since the use of mechanical sorting has been adopted for decontamination (under the umbrella of the RAWFILL project, https://www.nweurope.eu/projects/project-search/supporting-a-new-circular-economy-for-raw-materials-recovered-from-landfills/), a remediation technique was adopted for the treatment of fine fractions. The method was to dispose of wastes in landfill sites. The resumption of the studies previously carried out, the analysis and the synthesis of the numerous measures were necessary in order to acquire a detailed knowledge of the site. The observation of pollutants such as metal(loid)s, PCBs and hydrocarbons as well as their respective locations were at the origin of a new site zoning. The identification of the hydrocarbon pit justified the direct shipment of a waste zone to a hazardous waste storage facility without passing through the sorting chain and thus eliminating the main source of pollution. The recommendations for the site confirmed zoning in the preliminary project through additional surveys and analyzes as well as proposed representative sampling methods in view of an analytical follow-up during construction. The precise zoning of the old landfill has led to identify sulfate pollution from the landfill due to the presence of plaster.

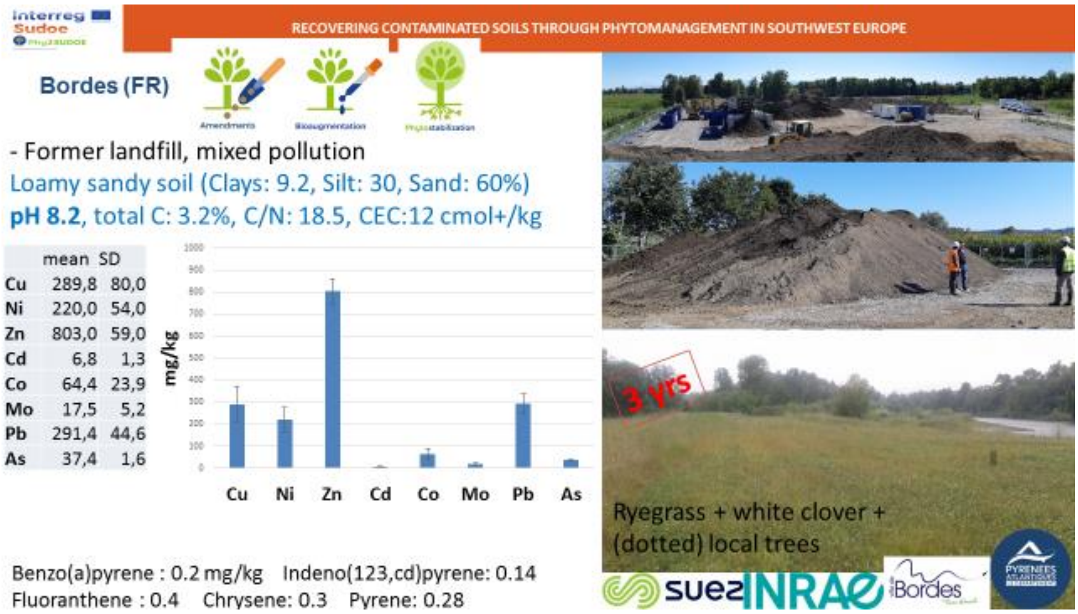
The future land use is to recreate a local meadow reinforced by a riparian forest.



Location of the Bordes site and zoning © CD64/ Bordes town, Suez Lyre

● **Soil properties**:

It is a technosol rebuilt to cover the landfill; soil pH: alkaline (7.9 – 8.4). The loamy anthroposol displays an alkaline soil pH, and Zn/Pb/Cu/Ni/Cd concentrations in excess. The soil contaminants were listed in the following table.



Soil characterization at the Bordes site ©Mench INRAE/ CD64

Pollutant	Data	Total concentration in the soil (mg/kg DW)	Legal Limit*
As		28 - 96	
Cd		5 - 32	
Cr		67 - 409	
Cu		239 - 1000	

Hg		0.5 – 2.9	
Ni		119 - 710	
Pb		187 - 461	
Zn		621 - 1490	
∑ BTEX		<0.05 – 0.07	
∑ Polychlorobiphenyls (PCB)		< 0.01 – 1.19….	
∑ PAH		0.1 – 4.5	

* no maximum permitted concentration (MPC) for total soil metal(loid)s in France. Risk assessment is site-specific (Info Terre 2018) and mainly based on bioavailable soil metal(loid)s as compared to background values for uncontaminated soils from the same soil series (or with similar soil texture) and bioassays (using ecotoxicological battery in line with current and future land use)
© CD64 /Bordes town, Suez Lyre

● Phytomanagement option /plant assembly

The objective was to phytostabilize in the soil the metal(loid)s in excess, to promote the rhizo/biodegradation of organic contaminants, to avoid/reduce the pollutant linkages, and to safely and sustainably increase the food web complexity.

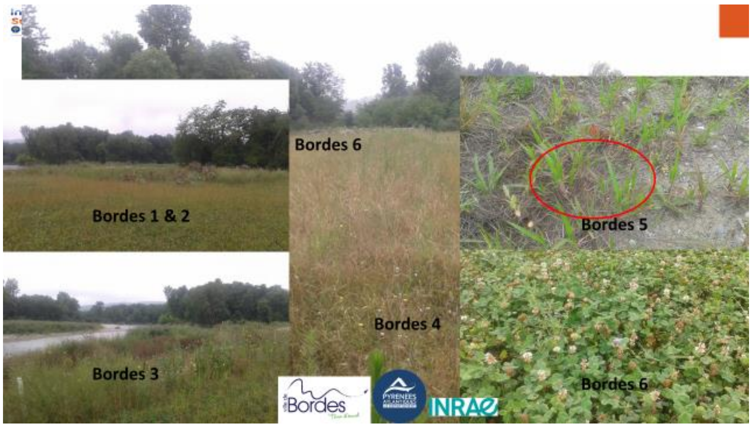
.

Local (native) grassy plant species (from northern Bearn meadows) and those used for green manure (rye, white clover and ryegrass) and local tree species were implemented;
The seed bank was increased by hay transfer (the succession should result in a Riparian forest).

Plant species used: *Festuca rubra, Trisetum flavescens, Anthoxanthum odoratum, Poa pratensis, Trifolium pratensis, Vicia sepium, Silene vulgaris, Plantago lanceolata, Holcus lanatus,* and *Agrostis capillaris*
Tree species: *Fraxinus excelsior, Acer platanoides, Sorbus torminalis, Alnus glutinosa, Corylus avellana, Salix caprea,* and *Salix viminalis*.

Implementation of the green capping; bioaugmentation with soil and hay transfer, transplantation of local trees © CD64 /Bordes town, Suez Lyre

Map of the tree plantations © Paille-Barrère /CD64



Vegetation cover at various plots of the Bordes site in 2022 ©Mench/INRAE/CD64

- **Success /limits**

- Soil amendments, bioaugmentation, phytostabilization and phytoextraction of metal(loid)s, and rhizo/biodegradation of organic contaminants were investigated in 2022 as well as soil biological activities;

- The vegetation cover rate is excellent (nearly 100%); no erosion was detected. This creates a habit colonized by insects such as pollinators;

-The site is gradually returning to nature in the form of a meadow reinforced by a riparian forest.

- The monitoring of the grassland composition, the seed banks, and soil microbial communities evidences a clear rehabilitation and progress in soil quality and biodiversity in line with metal(loid) phytostabilization and dissipation of residual organic compounds.

- The diversity of soil seed banks is increasing. The diversity of plant community was enhanced by hay and soil transfer.

- no metallophyte was present in the plant community on site;

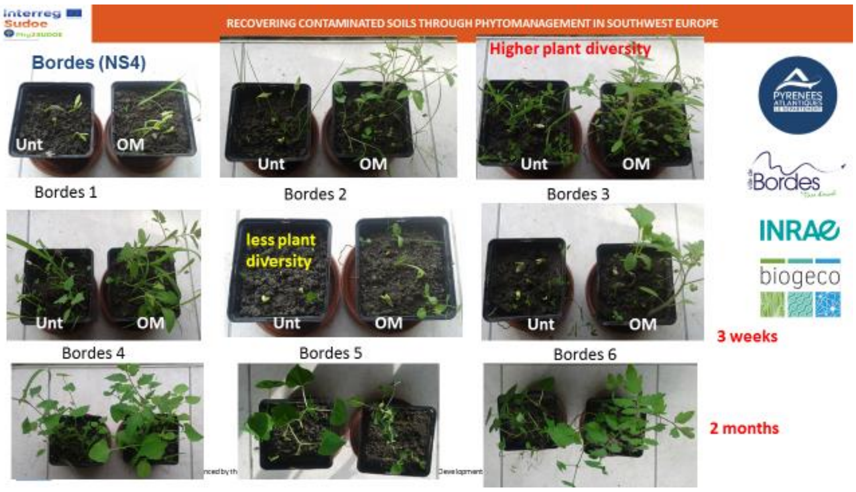
- The pot experiments carried out in 2022 with topsoils collected at this site has evidenced no remaining visible phytotoxicity on the shoots (on a sensitive plant species such as dwarf beans)

- Soil seed banks displayed a relatively high biodiversity for 5 out of 6 contaminated soils collected at 6 different plots.

- The addition of compost stimulated the plant growth and the diversity of the plant community.

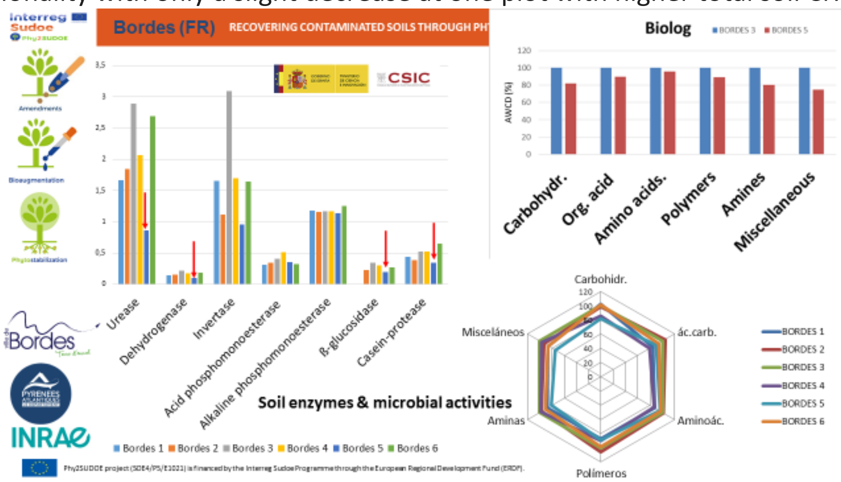
Screening of plant community and soil sampling to investigate microbial community at the Bordes site © Mench/INRAE - CD64

Butterfly on white clover on site and study of seed banks and soil phytotoxicity for the Bordes site © Mench/INRAE

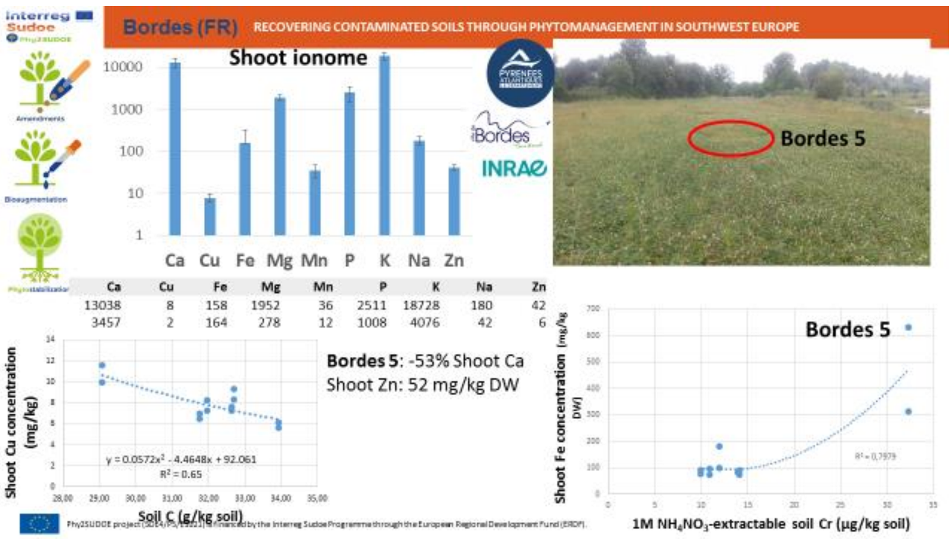


Plant testing of the Bordes soils with and without compost addition © Mench/INRAE

- Soil microbial strains including plant-growth promoting bacteria ones were identified and stored by CSIC and UCP-ESB
- Only one out of six plots showed lower soil enzyme activities (determined by the CSIC partner on INRAE collected soils). The other ones were satisfying. The Biolog tests (done by CSIC) indicated a good biological functionality with only a slight decrease at one plot with higher total soil Cr.



Soil enzymes and microbial activities in the Bordes soil samples (INRAE/CSIC/CD64/Bordes town)



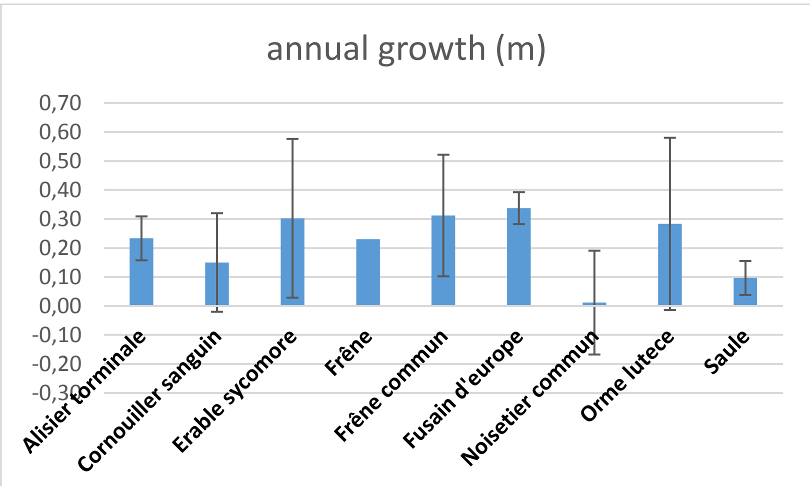
Shoot ionome of the vegetation cover at the Bordes sites © Mench INRAE/CD64

The analysis of shoot ionome of the (washed) grassy species showed the macronutrients (Ca, Mg, K, and P) in the common ranges. Micronutrients (Zn, Cu, Mn and Fe) concentrations ranged also in the common values without excess (showing mainly a metal-excluder pattern). Shoot Cu concentration decreased as soil organic C enhanced as expected. In the Bordes 5 plot, shoot Ca concentration was halved and shoot Fe concentration was in excess, which matched with an increase in 1M NH_4NO_3-extractable soil Cr and some purple symptoms on the leaf sheath of *Holcus lanatus*.

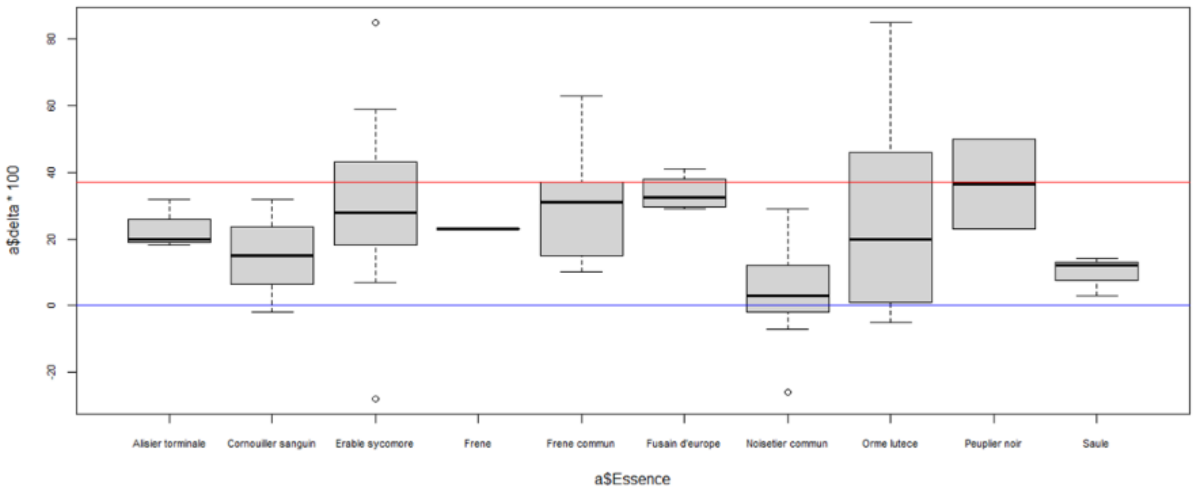
Recording of the tree growth in 2023 © Paille-Barrere/CD64

Tree growth: the alders (*A. glutinosa*) had withered following the 2022 heat wave. They were replaced by black poplars (*P. nigra*).

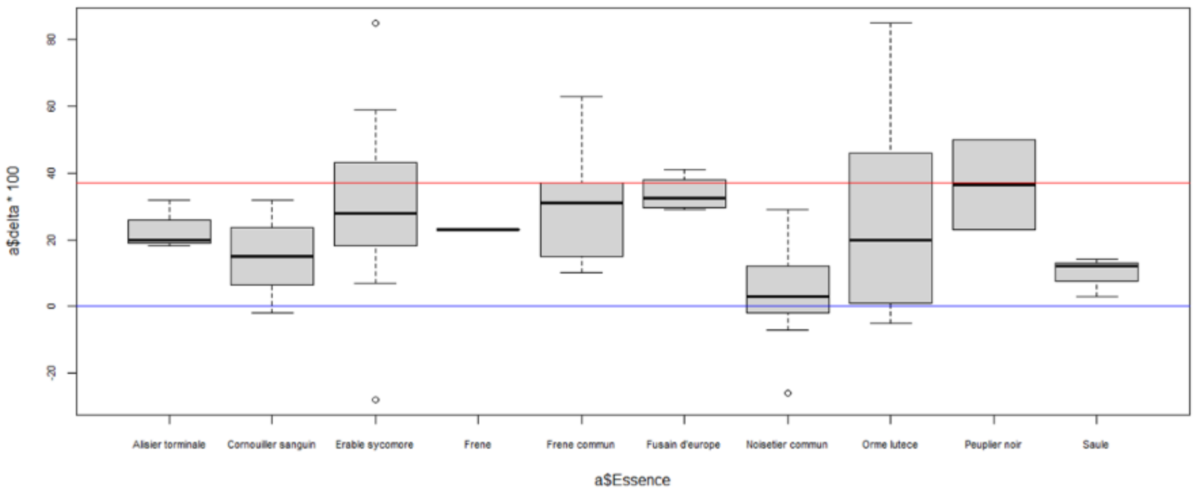
Fraxinus excelsior, Acer platanoides, Sorbus torminalis, Alnus glutinosa, Corylus avellana, Salix caprea, and *Salix viminalis*.

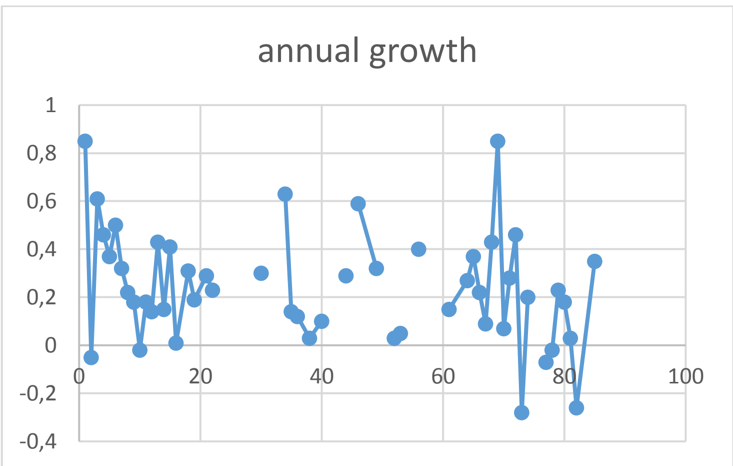


Annual growth (m) depending on tree species (March 2023) ©Paille-Barrere/CD64



The higher mean values for the tree growth were recorded for *A. platanoides (*Erable sycomore*), F; excelsior (*Frêne commun*), Fraxinus (*Frêne*), Euonymus europaeus (*Fusain d'Europe*)* and *Ulmus lutèce (*Orme lutèce*).* However the influence of the tree species was insignificant (p =0.4). The influence of the zones was suggested as the tree growth was lower in the zone 3 (more plant-plant competition with a dense and diverse herbaceous community, high seed bank) and in the zone 5 (1M NH_4NO_3-extractable soil Cr in excess detected and visible purple symptoms on *H. lanatus*, no white clover).





Tree growth according to their site location (see the map): zone 3 (n° 21-29), and zone 5 (n°53-60)

NS5 - Bandeira:

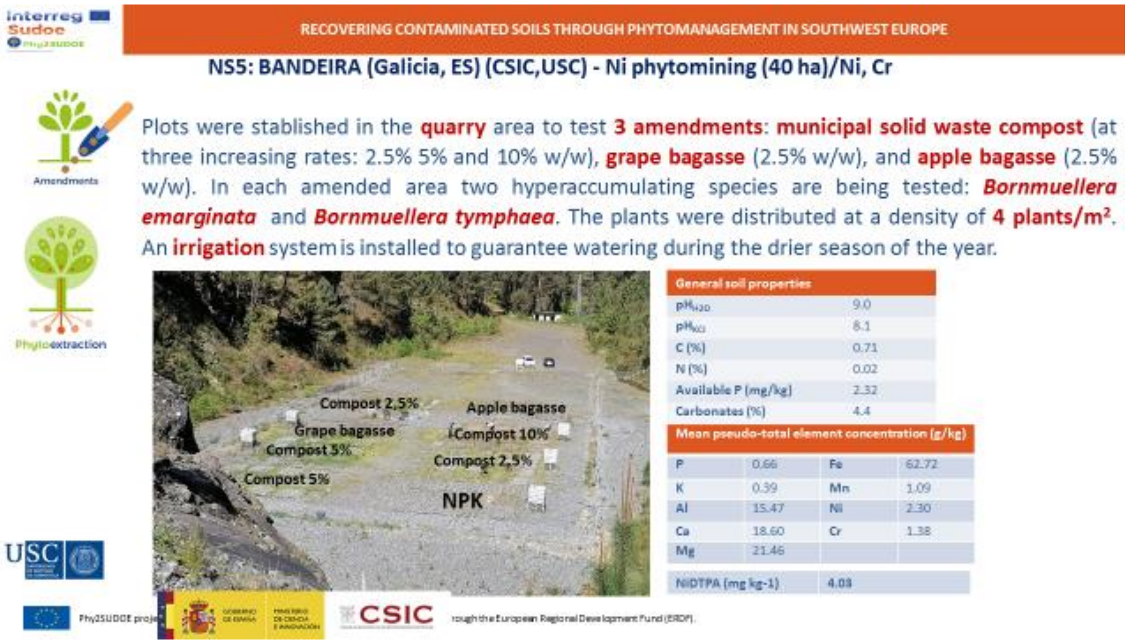
● **Implementation**: This site is an abandoned bare serpentine quarry (40 ha) in Galicia. It is located in the Melide-Serra do Careón geological complex, which represents one of the three main serpentine outcrops of the Iberian Peninsula. It is characterised climatically by a high precipitation (annual mean 1375 mm) and mild temperatures (annual mean 12.6 °C). The active opencast mine is embedded in a substrate of amphibolites and serpentines, covering an area of 40 ha and is dedicated to the extraction of serpentinized peridotite for the production of gravel for construction and ballast for railway tracks. The generated sterile material is accumulated in spoil heaps and these cover an area of around 3.4 km^2.

Location, field trial and conceptual model for the Bandeira site © CSIC-USC

● **Soil properties**

The soil derived from the spoil material – classified as Spolic Technosol (IUSS Working Group WRB, 2014) – is shallow and gravelly, with a poor structure, low water retention capacity and is mostly bare of vegetation, with *Cortaderia selloana* as the only successful colonising plant species. The mine-soil is characterised by a basic pH (7.8) and poor fertility, which is reflected in the low total C and N content and nutrient concentrations (available P and K).

The soil is derived from the ultramafic rock exploited in the quarry, with high total soil Ni but with relatively low Ni availability in comparison with more developed ultramafic soils.



Initial physico-chemical soil properties at the Bandeira site © CSIC-USC

Quarry Soil		
	Mean	SE
pH (H2O)	9,0	0,0
pH (KCl)	8,1	0,0
Total C (%)	0,7	0,0
Total N (%)	0,0	0,0
P Olsen (mg/kg)	2,3	0,8
CEC (cmolc/kg)	13,0	1,2
Ca	9,2	0,8
Mg	3,4	0,3
K	0,2	0,0
Pseudo-total metal concentration		
Co (mg/kg)	84,2	6,1
Cr (mg/kg)	1378,5	104,7
Cu (mg/kg)	21,9	2,6
Fe (mg/kg)	62722,0	4946,0
Mn (mg/kg)	1093,9	69,0
Ni (mg/kg)	2302,0	194,6
Pb (mg/kg)	7,7	0,9
Zn (mg/kg)	32,4	1,5

- **Phytomanagement options /plant assembly**

The experimental plots are located in one of the spoil heap of this quarry.

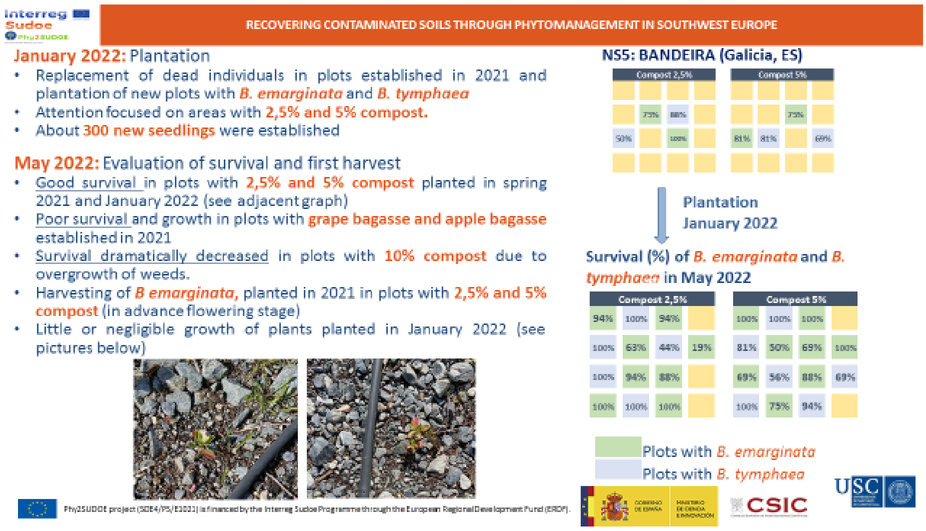
Based on previous pot experiments and field trials dating back to 2015 (using various nickel hyperaccumulators: *Bornmuellera emarginata*, *B. tymphaea*, *Odontarrhena serpyllifolia*, *Odontarrhena chalcidica* and *Noccaea caerulescens*), the phytomanagement option was based on phytomining using two Mediterranean Ni hyperaccumulators: *Bornmuellera emarginata* and *B.*

tymphaea, native from Greece. The objective was to phytoextract Ni from the soil and to produce Ni ore from the biomass processing.

Plots were stablished in the quarry area to test 3 amendments for promoting plant growth, shoot biomass production, and Ni-phytomining: municipal solid waste compost (at three increasing rates: 2.5% 5% and 10% w/w), grape bagasse (2.5% w/w), and apple bagasse (2.5% w/w). In each amended area two Ni-hyperaccumulating plant species are being tested: *Bornmuellera emarginata* and *B. tymphaea*. The plants were distributed at a density of 4 plants/m^2. An irrigation system was installed to guarantee watering during the drier season of the year.

Since January 2022, dead individuals were replaced in plots established in 2021. New plots were planted with *B. emarginata* and *B. tymphaea*. Attention was focused on areas with 2.5% and 5% of compost. About 300 new seedlings were established.

Since May 2022, survival rate was evaluated and first shoot harvest realized. Good survival rate was evidenced in plots with 2.5% and 5% compost planted in spring 2021 and January 2022. Poor survival rate and plant growth occurred in plots amended with grape bagasse and apple bagasse established in 202. The survival rate was dramatically decreased in plots with 10% compost due to overgrowth of weeds. The *B emarginata*, planted in 2021, were harvested in plots with 2.5% and 5% compost (in advance flowering stage). Currently little or negligible growth of plants was noticed for those planted in January 2022. *Noccaea caerulescens* was tested in the plots by direct sowing in the field.



Scheme of field plots implemented at the Bandeira site © CSIC-USC

● Success / limits:

Both *Bornmuellera* species successfully established in plots amended with 2.5% and 5% of municipal solid waste compost. Their mortality however was high in plots amended with 10% compost, grape bagasse or apple bagasse.

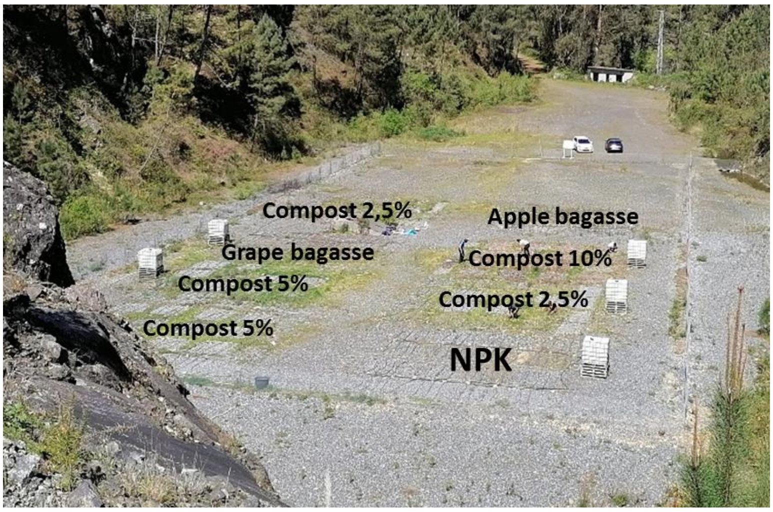
Plants established in spring 2021 were harvested in spring 2022. The biomass of *B. emarginata* generally reached 300 kg ha^{-1}. The Ni yield peaked with 2.5% compost amendment (about 1kg Ni ha^{-1}) as compared to soils receiving 5% compost (up to 0.4 kg Ni ha^{-1}).

Plant resprout after harvest was better in the low compost rate-amended area. Biomass (up to 135 tm ha^{-1} with 5% compost amendment) and Ni yield (about 0.1 kg Ni ha^{-1}) obtained with *B. tymphaea* were lower than with *B emarginata*.

The plants established in January 2022 showed good survival after 10 months, especially in the low compost rate-amended area (65% for *B emarginata* and 72% for *B tymphaea*), and will be harvested in spring 2023.

This phytomanagement improves soil properties related to fertility and the provision of other ecosystem services.

Limits are water supply and distribution along the year in line with climate change (heatwaves, drought) and the low water holding capacity of this soil. Implementation of the irrigation system was relevant.



NS5. Field trial at the Bandeira site, Spain (© B. Rodriguez *et al.*, CSIC)

Table NS5. Soil properties after phytomanagement

Data	Plot	Replicate	Sample	pH		LECO		P.Olsen mg/Kg P
				in H2O	in KCl	C %	N %	
03/06/2021	Compost 2,5%	1	COM 2,5 no plantado-1	7,19	6,48	1,90	0,10	181,87
		2	COM 2,5 no plantado-2	7,21	6,47	1,92	0,14	161,39
		3	COM 2,5 no plantado-3	7,23	6,53	1,90	0,13	181,94
		MEAN		7,21	6,49	1,91	0,12	175,07
	Compost 5% -Plot 1	1	COM 5 (2016)no plantado-1	6,73	6,06	3,86	0,38	284,54
		2	COM 5 (2016)no plantado-2	6,74	6,08	3,88	0,38	280,70
		3	COM 5 (2016)no plantado-3	6,82	6,07	3,82	0,39	297,95
		MEAN		6,76	6,07	3,85	0,38	287,73
	Compost 5% - Plot 2	1	COM 5 (2017)no plantado-1	6,97	6,16	4,01	0,40	292,58
		2	COM 5 (2017)no plantado-2	7,01	6,19	4,09	0,38	286,47
		3	COM 5 (2017)no plantado-3	6,98	6,20	3,97	0,42	257,60
		MEAN		6,99	6,18	4,02	0,40	278,88

Data	Plot	Replicate	Sample	NH4Cl Extraction															
				Al (mg/kg)	Ca (mg/kg)	Co (mg/k	Cr (mg/kg)	Cu (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Na (mg/kg)	Ni (mg/kg)	Al (cmol+/kg)	Ca (cmol+/kg)	K (cmol+/kg)	Mg (cmol+/kg	Na (cmol+/kg)	CEC (cmol+/kg)
03/06/2021	Compost 2,5%	1	COM 2,5 no plantado-1	1,36	719,37	0,15	0,00	0,61	48,15	486,26	1,20	8,53	1,40	0,02	3,59	0,12	4,00	0,04	7,77
		2	COM 2,5 no plantado-2	0,79	693,82	0,08	0,04	0,69	41,02	463,88	1,08	5,79	1,19	0,01	3,46	0,10	3,82	0,03	7,42
		3	COM 2,5 no plantado-3	1,09	659,30	0,14	0,07	0,78	50,14	431,23	1,08	0,00	1,32	0,01	3,29	0,13	3,55	0,00	6,98
		MEAN		1,08	690,83	0,12	0,04	0,69	46,44	460,46	1,12	4,77	1,30	0,01	3,45	0,12	3,79	0,02	7,39
	Compost 5% -Plot 1	1	COM 5 (2016)no plantado-1	1,86	1013,13	0,11	0,05	0,76	89,85	549,36	3,41	0,00	1,98	0,02	5,06	0,23	4,52	0,00	9,83
		2	COM 5 (2016)no plantado-2	1,51	967,45	0,10	0,10	0,70	93,13	525,76	3,17	0,00	1,85	0,02	4,83	0,24	4,33	0,00	9,41
		3	COM 5 (2016)no plantado-3	2,13	1117,84	0,08	0,04	1,01	105,81	603,46	3,72	5,22	2,40	0,02	5,58	0,27	4,96	0,02	10,86
		MEAN		1,83	1032,81	0,10	0,06	0,82	96,26	559,53	3,43	1,74	2,08	0,02	5,15	0,25	4,60	0,01	10,03
	Compost 5% - Plot 2	1	COM 5 (2017)no plantado-1	2,16	1253,98	0,18	0,02	0,74	71,60	539,47	2,82	5,60	1,79	0,02	6,26	0,18	4,44	0,02	10,93
		2	COM 5 (2017)no plantado-2	1,95	1093,71	0,22	0,11	0,82	68,36	470,78	2,36	3,67	1,73	0,02	5,46	0,17	3,87	0,02	9,54
		3	COM 5 (2017)no plantado-3	1,99	1212,62	0,21	0,06	0,96	73,58	534,20	2,51	4,97	1,62	0,02	6,05	0,19	4,39	0,02	10,68
		MEAN		2,04	1186,77	0,20	0,06	0,84	71,18	514,82	2,56	4,75	1,71	0,02	5,92	0,18	4,24	0,02	10,38

Data	Plot	Replicate	Sample	DTPA									
				Ca (mg/kg)	Co (mg/kg)	Cu (mg/k	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Zn (mg/kg)	Al (mg/kg)
03/06/2021	Compost 2,5%	1	COM 2,5 no plantado-1	848,75	0,04	3,53	45,91	11,53	335,14	2,99	13,80	6,26	0,00
		2	COM 2,5 no plantado-2	837,49	0,06	3,68	47,37	12,74	330,04	2,98	13,83	6,22	0,00
		3	COM 2,5 no plantado-3	838,88	0,03	3,53	44,32	11,23	320,83	2,85	13,11	6,13	0,00
		MEAN		841,71	0,04	3,58	45,87	11,83	328,67	2,94	13,58	6,20	0,00
	Compost 5% -Plot 1	1	COM 5 (2016)no plantado-1	658,83	0,10	8,04	97,96	8,97	263,79	6,20	34,72	9,32	4,59
		2	COM 5 (2016)no plantado-2	666,45	0,08	8,31	98,83	8,25	264,05	6,23	35,01	9,49	4,67
		3	COM 5 (2016)no plantado-3	670,03	0,07	8,75	100,98	7,62	265,05	6,63	36,54	9,87	4,65
		MEAN		665,10	0,08	8,37	99,26	8,28	264,30	6,35	35,43	9,56	4,64
	Compost 5% - Plot 2	1	COM 5 (2017)no plantado-1	709,38	0,09	8,73	116,68	8,40	243,25	5,73	24,33	19,81	1,80
		2	COM 5 (2017)no plantado-2	709,05	0,06	8,92	119,25	8,08	244,86	5,74	24,74	19,79	1,80
		3	COM 5 (2017)no plantado-3	718,33	0,11	8,69	116,24	7,38	250,33	5,69	24,77	19,50	1,71
		MEAN		712,25	0,09	8,78	117,39	7,96	246,15	5,72	24,61	19,70	1,77

Data	Plot	Replicate	Sample	Pseudo-total metals (mg/Kg)												
				Ca (mg/kg)	Co (mg/kg)	Cu (mg/k	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	P (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Al (mg/kg)	Cr (mg/kg)
03/06/2021	Compost 2,5%	1	COM 2,5 no plantado-1	3289,94	56,94	49,74	49743,37	845,54	144363,85	593,60	1799,00	1236,55	4,21	64,94	14740,03	973,71
		2	COM 2,5 no plantado-2	3178,35	56,66	49,64	49400,60	802,32	146863,28	583,44	1733,02	1132,82	8,52	64,28	14064,05	945,15
		3	COM 2,5 no plantado-3	3115,58	61,84	56,94	49696,94	832,10	145796,69	604,24	1775,08	1197,37	5,74	62,30	14479,08	965,37
		MEAN		3194,62	58,48	52,11	49613,64	826,66	145674,60	593,76	1769,03	1188,91	6,16	63,84	14427,72	961,41
	Compost 5% -Plot 1	1	COM 5 (2016)no plantado-1	4084,95	49,50	61,03	43235,67	1855,78	125567,07	527,25	1343,44	2145,91	4,54	77,08	19507,36	771,79
		2	COM 5 (2016)no plantado-2	4134,43	49,59	65,64	47708,75	2066,52	128787,40	524,59	1380,31	2154,60	8,40	76,84	20348,98	827,51
		3	COM 5 (2016)no plantado-3	4216,57	49,52	71,88	43768,02	1734,67	126949,71	533,48	1401,24	2296,06	11,50	79,20	19494,77	815,29
		MEAN		4145,32	49,54	66,18	44904,15	1885,66	127101,39	528,44	1375,00	2198,86	8,14	77,71	19783,70	804,86
	Compost 5% - Plot 2	1	COM 5 (2017)no plantado-1	4330,99	52,30	68,86	47084,87	1161,59	131767,72	515,84	1470,64	2528,69	11,57	117,64	17242,85	866,03
		2	COM 5 (2017)no plantado-2	4261,35	54,50	74,24	47396,86	1226,65	135541,84	524,49	1503,32	2551,89	10,68	120,03	17747,55	896,96
		3	COM 5 (2017)no plantado-3	4337,93	51,71	74,59	46759,89	1270,93	132119,92	537,02	1522,61	2600,15	14,19	120,79	17662,32	926,01
		MEAN		4310,09	52,84	72,56	47080,54	1219,72	133143,16	525,78	1498,86	2560,24	12,15	119,48	17550,91	896,33

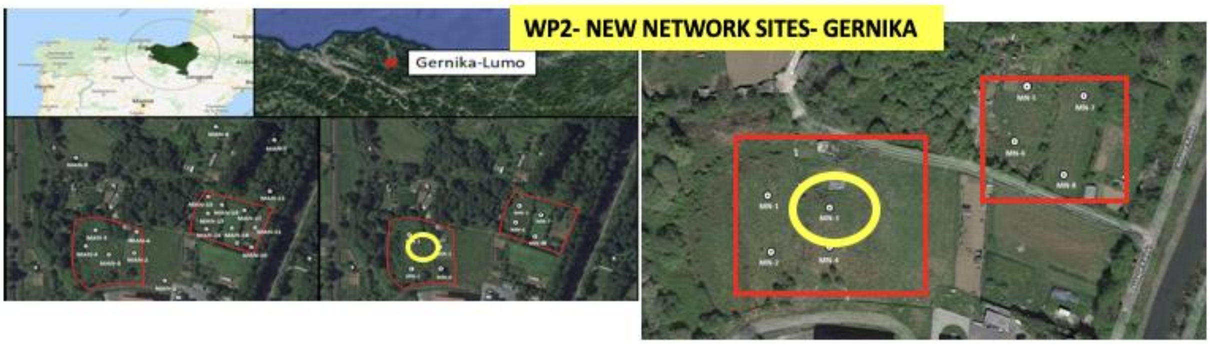
Data	Plot	Replicate	Sample	Sr(NO3)2 Extraction								
				Ca (mg/kg)	Co (mg/kg)	Cu (mg/k	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	P (mg/kg)
03/06/2021	Compost 2,5%	1	COM 2,5 no plantado-1	388,33	0,02	0,00	0,59	22,74	378,29	0,06	0,20	1,71
		2	COM 2,5 no plantado-2	373,00	0,04	0,00	0,38	21,02	358,11	0,05	0,22	1,71
		3	COM 2,5 no plantado-3	393,39	0,04	0,09	0,78	23,84	379,32	0,05	0,20	1,90
		MEAN		384,90	0,03	0,03	0,58	22,54	371,90	0,05	0,21	1,77
	Compost 5% -Plot 1	1	COM 5 (2016)no plantado-1	463,40	0,02	0,03	0,55	25,87	388,96	0,32	0,50	3,76
		2	COM 5 (2016)no plantado-2	462,08	0,02	0,06	0,39	25,52	400,01	0,32	0,46	3,95
		3	COM 5 (2016)no plantado-3	442,04	0,02	0,07	0,57	23,48	377,62	0,30	0,42	3,52
		MEAN		455,84	0,02	0,05	0,50	24,96	388,87	0,31	0,46	3,74
	Compost 5% - Plot 2	1	COM 5 (2017)no plantado-1	518,55	0,03	0,10	0,63	23,11	357,82	0,16	0,30	2,21
		2	COM 5 (2017)no plantado-2	549,90	0,02	0,36	0,98	20,81	369,21	0,18	0,30	1,83
		3	COM 5 (2017)no plantado-3	515,60	0,03	0,00	0,30	21,38	353,70	0,16	0,33	2,36
		MEAN		528,02	0,03	0,15	0,64	21,77	360,25	0,17	0,31	2,13

Table NS5. Shoot ionome of plants harvested at the Bandeira site

				concentration of metals in plant									% survival of vegetation	Biomass kg/ha	kg Ni/ha
Data	Plot	Plant	Sample	Ca (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	P (mg/kg)			
06/05/2022	Compost 2,5%	*B. emarginata*	com 2,5 Le1 Ba	11009,8	4,3	7,1	64,2	15772,7	1614,3	12,6	2263,5	2668,9		411	0,93
			com 2,5 Le2 Ba	13524,2	3,7	6,9	24,9	16488,9	2145,6	14,4	3030,7	2399,4		510	1,54
			com 2,5 Le3 Ba	8369,5	8,4	7,1	32,4	17854,3	2160,7	20,1	1807,1	2095,2		302	0,55
			MEAN	**10967,8**	**5,5**	**7,0**	**40,5**	**16705,3**	**1973,5**	**15,7**	**2367,1**	**2387,8**	**75,5**	**407,8**	**1,01**
		B. tymphaea	com 2,5 Bt1 Ba	12826,9	12,0	11,7	103,5	18896,2	1976,9	40,6	2467,7	4709,6		37	0,09
			com 2,5 Bt2 "ch	12280,4	5,2	10,4	183,6	15887,9	1663,0	34,4	3125,5	4474,9		8	0,02
			com 2,5 Bt3 "ve	19122,0	11,1	19,6	53,9	23793,5	1774,6	39,5	5559,2	5903,5		19	0,10
			MEAN	**14743,1**	**9,4**	**13,9**	**113,6**	**19525,9**	**1804,8**	**38,2**	**3717,4**	**5029,3**	**44**	**21,2**	**0,07**
	Compost 5% -Plot 1	*B. emarginata*	com 5 2016 Le1	14439,1	2,2	7,9	42,8	23235,1	2445,2	22,6	1690,7	3689,2		77	0,13
			com 5 2016 Le2	9541,9	1,2	12,6	29,8	23672,2	1762,2	16,3	1611,8	3007,8		46	0,07
			com 5 2016 Le3	8282,9	2,3	9,7	26,9	11028,9	1276,8	22,2	1349,6	2457,4		269	0,36
			MEAN	**10754,6**	**1,9**	**10,1**	**33,2**	**19312,1**	**1828,1**	**20,4**	**1550,7**	**3051,4**	**24,2**	**130,91**	**0,19**
		B. tymphaea	com 5 2016 Bt1	22389,5	2,0	22,7	68,8	32409,3	2803,4	35,3	1925,1	6409,2		56	0,11
			com 5 2016 Bt2	23908,2	0,4	22,0	100,5	29538,6	2794,0	52,9	739,1	6196,7		56	0,04
			com 5 2016 Bt3	19535,0	7,9	18,7	88,3	25925,0	2542,7	69,1	4391,8	5985,6		29	0,13
			MEAN	**21944,2**	**3,4**	**21,1**	**85,9**	**29291,0**	**2713,4**	**52,4**	**2352,0**	**6197,2**	**34,5**	**46,97**	**0,09**
	Compost 5% - Plot 2	*B. emarginata*	com 5 2017 Le1	12305,2	4,0	9,5	49,8	14099,2	1841,8	20,0	716,8	3606,8		367	0,26
			com 5 2017 Le2	13108,8	2,5	17,8	114,8	11066,6	1860,8	27,9	767,0	3028,7		529	0,41
			com 5 2017 Le3	16309,0	10,8	10,6	49,4	12871,1	1478,2	44,6	1357,8	4327,7		293	0,40
			MEAN	**13907,7**	**5,7**	**12,6**	**71,3**	**12679,0**	**1726,9**	**30,8**	**947,2**	**3654,4**	**69**	**396,52**	**0,36**
		B. tymphaea	com 5 2017 Bt1	26655,2	0,7	13,8	64,4	17529,6	3079,3	47,4	555,9	7237,1		91	0,05
			com 5 2017 Bt2	28521,6	2,2	15,3	91,3	22126,0	2494,3	65,5	620,8	5644,0		160	0,10
			com 5 2017 Bt3	20903,7	0,6	17,7	63,3	12713,7	1518,0	48,4	646,0	5082,5		156	0,10
			MEAN	**25360,1**	**1,2**	**15,6**	**73,0**	**17456,4**	**2363,9**	**53,8**	**607,6**	**5987,9**	**62,5**	**135,42**	**0,08**

NS6 - Gernika: In the Basque Country Government inventory (165/2008 decree, relative to soils supporting potentially polluting activities or facilities), 1277 landfills are inventoried; including spilling points. In that inventory, Landfill 17 can be found, with 48046-00181 code. This landfill is located in the Biosphere Reserve of Urdaibai (UNESCO, 1984) in the vicinity of Gernika town (43°19'28.9"N 2°40'30.9"W).The landfill 17, which has an inventory area of 3.38 ha and 16,000 m^2, was used for decades as disposal point receiving sewage sludge (used as fertilizer) from a WWTP. Several pollutants from local industry (metals, PAHs, pesticides…) had been scattered along the Landfill (Fig NS6. 1).

● **Implementation**: this site is a former landfill with uncontrolled application of sewage sludge from an urban wastewater treatment plant.



Main pollutants in initial characterization: metals (Cd, Cr, Pb, Ni); organic (benzo(a)pyrene, dieldrin) other contaminants (As, Zn, PCBs) not surpassing reference levels

		MN1	MN2	MN3	MN4	MN5	MN6	MN7	MN8
Cadmium	mg/kgms	12	11	26	16	4,9	4,3	7,6	10
Chromium	mg/kgms	130	120	320	170	47	44	180	300
Lead	mg/kgms	62	60	150	96	38	41	66	89
Nickel	mg/kgms	53	48	100	64	28	28	78	120
BaP	mg/kgms	0,04	0,04	0,1	0,08	0,03	0,04	0,08	0,23
Dieldrin	µg/kgms	14	19	58	5,9	<1	<1	2,9	9,3
Pollution index		7,45	7,64	19,76	10,02	3,39	3,78	7,97	17,76

Figure NS6.1. Sites description and characterization of pollutants

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

In February 2017, the company GEYSER HPC carried out a Risk Assessment considering the intake of metals from the vegetables grown in these soils and used as food crops. The report concluded that the presence of contaminants such as dieldrin or benzo(a)pyrene did not completely eliminate the uncertainties and recommended an extension of the sampling of vegetables with a complete analytical program in order to correctly assess the situation of the site regarding the risks for the users of the area and to recover this site. In this context this emplacement was included as a new emplacement as part of the Phy2sudoe network.

● **Soil properties**: the soil displayed a mixed contamination with Cd, Cr, Ni, Pb, dieldrin, benzo(a)pyrene, benzofluoranthene and benzo anthracene in excess.

After a complete physicochemical characterization of soil properties in several plots of the emplacement, it was evidenced that the soil displayed a mixed contamination with Cd, Cr, Ni, Pb, dieldrin and benzo(a)pyrene surpassing reference levels for contaminated soils. Pollution index ranged from 7.45 to 29.76. The most polluted point, with the highest levels of all contaminants, was MN3 with very high content of dieldrin 58 µg/Kg DW and a pollution index of 29.76 (Figure NS6.1).

Several ecotoxicity assays were carried out with earthworms (cocoon and juvenile numbers, biomass, coelomocyte (acute and chronic) toxicity assays), plants (germination and root elongation bioassay with cucumber, onion and lettuce) and soil microorganism (soil respiration, biomass and functional diversity).

● **Phytomanagement options:**

In this scenario the objective was to implement at the worst site (MN3) several biological strategies to remediate the emplacement and to assess dissipation of contaminants and improvements of soil ecotoxicity. Soil movements were done for landscape integration and elimination of spontaneous vegetation was carried out. A perimeter fence was placed to avoid affection to the site by big herbivores (wild boards), which were common animal species in the area.

The objective was to assess the efficacy of biological remediation technologies with plants, worms or microbes, alone and in combination. As shown in Figure NS6.2, various bioremediation options were established in a pilot project along one year: NT: Non-treated; E: earthworms (*Eisenia fetida*); B: bacteria (microbial consortium); P: plants (alfalfa); P+B: plants + bacteria; P+E: plants + earthworms; B+E: bacteria + earthworms; and P+B+E: plants + bacteria + earthworms. Seeds of *Medicago sativa* (a legume nitrogen fixing species) were sown in spring at a dose of 20 Kg/ha. We deposited 170 specimens of *E. fetida* (from a commercial retailer) per m^2 in autumn, and a second application next spring. In the plot inoculated with microbes, we supplied four applications (one in autumn and three in spring) of a consortium of *Burkholderia xenovorans* LB400 (a bacteria with catabolic activity that degrades aromatic compounds) and *Paenibacilus sp* (selected from contaminated soils and useful for degradation of organic compounds).

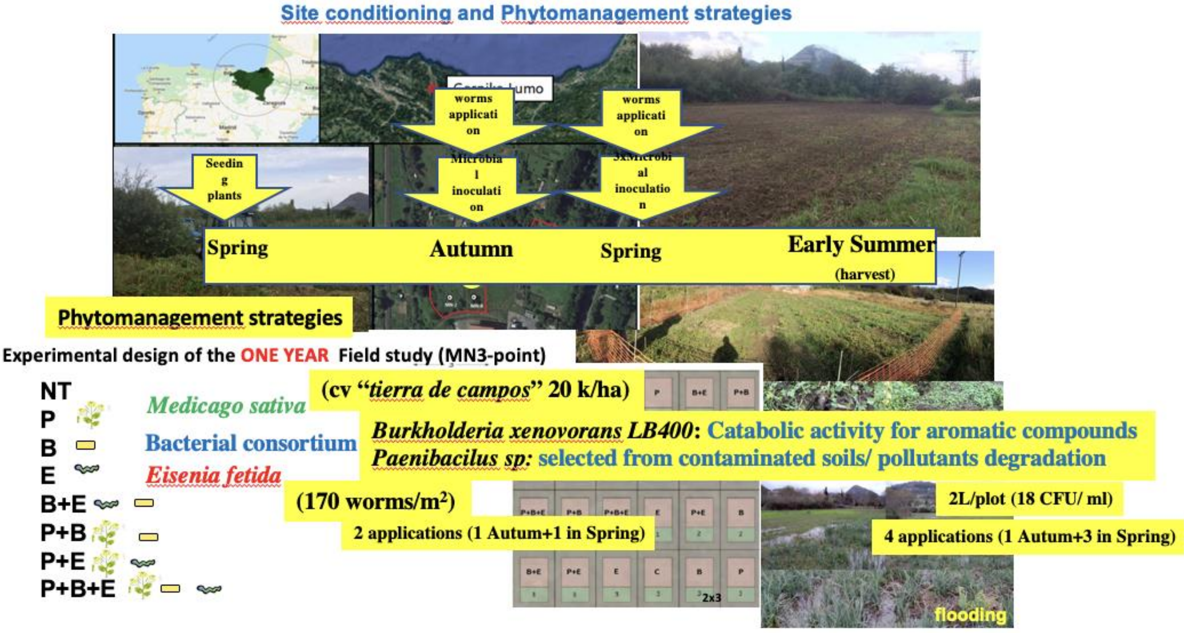


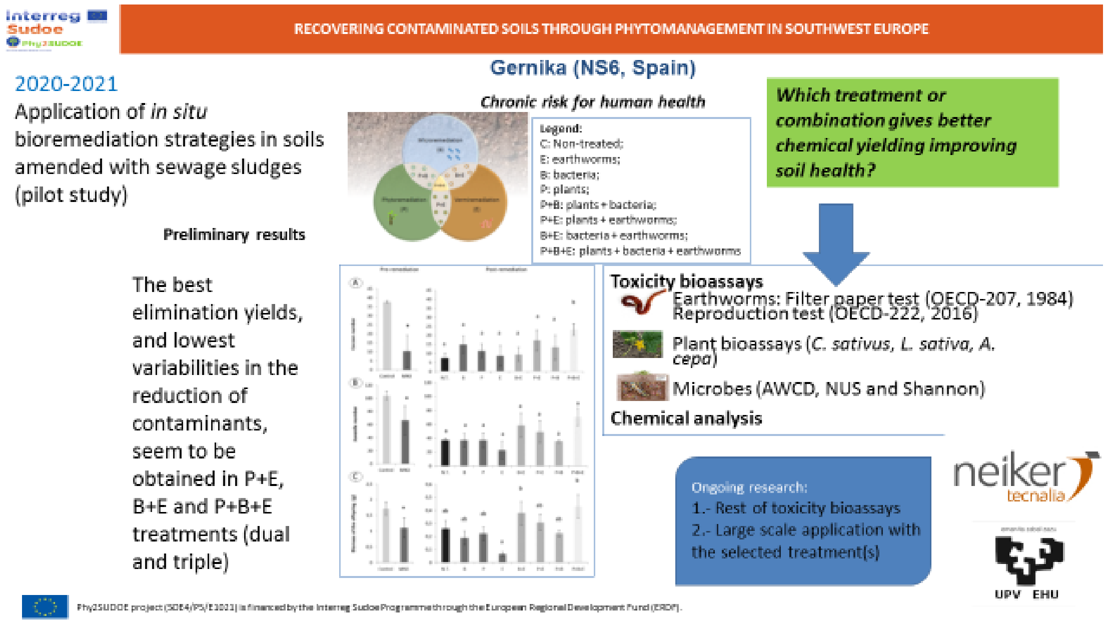
Figure NS6.2. Phytomanagement strategies implemented in Gernika.

Plant/microbe assembly: Various bioremediation options are established: C: Non-treated; E: earthworms (*Eisenia fetida*); B: bacteria (microbial consortium); P: plants (alfalfa); P+B: plants + bacteria; P+E: plants + earthworms; B+E: bacteria + earthworms; and P+B+E: plants + bacteria + earthworms. The best elimination yields, and lowest variabilities in the reduction of contaminants, seem to be obtained in P+E, B+E and P+B+E treatments (dual and triple).

● **Success / limits**

After one year, a great decrease in pollutant concentrations occurred in all treated plots (see the green background in Figure NS6.3). In fact, several pollutants decreased under the reference levels for contaminated soils. After one year, only Cd levels remained above reference levels for contaminated soils. Benzo(a) pyrene was very difficult to remove, and consequently only a 19-28 % of the initial levels were eliminated. However, the best result was in the case of the pesticide dieldrin that decreased well below reference levels for contaminated soils. The best elimination yields for the P+B+E treatment were: Dieldrin (between 50% and 78%), Metals (20–25%, Cd 15%–35%; Ni 24%–37%; Pb 15%–33%; Cr 7%–39%), Benzo(a)pyrene (19.5%–28%). Quantitative risk assessment is ongoing.

The most efficient phytomanagement option combines crop (e.g. alfalfa), bacterial consortium, and earthworms. It decreases both total soil dieldrin, Cd, Pb, and Cr in excess, leading to a partial recovery of soil health indicated by decreased toxicity for plants and worms.



Ecotoxicological tests and bioassays: earthworms, plants and bacteria © UPV/Neiker

Preparation of the Gernika field trial (© M. Soto et al., UPV)

Pollutants levels in soil in one year pilot assay

Concentrations (mg/kg soil) of cadmium, chromium, lead, nickel, dieldrin and Benzo(a)pyrene –B(a)P- in each subplot, after individual, dual and triple application of vermi-, phyto- and microremediation technologies. Mean values and standard deviations for each treatment are shown. Values in bold correspond to values exceeding Basque legislation threshold for polluted sites. E earthworms; B bacteria; P plants; P+B plants + bacteria; P+E plants + earthworms; B+E bacteria + earthworms; P+B+E plants + bacteria + earthworms.

Compound		Basque Thresholds	B Pre	B Post	P Pre	P Post	E Pre	E Post
Cadmium	mg/kgdw	**5**	**11.73 ± 3.4**	**9.7 ± 4.2**	**15.33 ± 3.8**	**12.1 ± 2.6**	**19.03 ± 10.5**	**11.9 ± 4**
Chromium	mg/kgdw	**200**	140 ± 10	131.9 ± 49.5	186.66 ± 35.1	160 ± 26.2	**236.6 ± 135.8**	164.3 ± 61.3
Lead	mg/kgdw	**120**	86.33 ± 3.2	73.6 ± 19.7	102 ± 17.1	80 ± 0.0	113 ± 50.9	80 ± 24
Nickel	mg/kgdw	**110**	60.66 ± 4.7	46 ± 12.4	69.33 ± 7.6	51.3 ± 5.7	78.33 ± 30.6	52.8 ± 14.7
BaP	mg/kgdw	**0.02**	**0.08 ± 0.01**	**0.06 ± 0.03**	**0.09 ± 0.02**	**0.06 ± 0.01**	**0.11 ± 0.09**	**0.06 ± 0.02**
Dieldrin	μg/kgdw	**10**	**13.26 ± 13.2**	5 ± 5.1	**33.33 ± 21.5**	8.1 ± 3.7	**49.5 ± 16.3**	8.2 ± 5.8

Compound		Basque Thresholds	P+E Pre	P+E Post	B+E Pre	B+E Post	P+B Pre	P+B Post
Cadmium	mg/kgdw	**5**	**19 ± 7**	**11.7 ± 4.1**	**18.2 ± 11.2**	**11.3 ± 5**	**12.5 ± 2.8**	**12.8 ± 0**
Chromium	mg/kgdw	**200**	**220 ± 79.4**	144.9 ± 44.2	**234 ± 134.7**	151.4 ± 68.7	156.66 ± 40.4	155.7 ± 0
Lead	mg/kgdw	**120**	115 ± 35	75.1 ± 21.9	115.66 ± 56.6	75.8 ± 26.6	89.33 ± 11	86.7 ± 0
Nickel	mg/kgdw	**110**	81 ± 20.8	49.8 ± 11.6	82.33 ± 37.5	52.1 ± 17.7	58.33 ± 8.5	51.7 ± 0
BaP	mg/kgdw	**0.02**	**0.09 ± 0.03**	**0.06 ± 0.03**	**0.07 ± 0.03**	**0.06 ± 0.02**	**0.06 ± 0.01**	**0.06 ± 0**
Dieldrin	μg/kgdw	**10**	**27.03 ± 22.6**	3.6 ± 3.5	**34.96 ± 31.1**	8.1 ± 7.6	**24.96 ± 22.3**	6.1 ± 0

Compound		Basque Thresholds	P+B+E Pre	P+B+E Post
Cadmium	mg/kgdw	**5**	**15.1 ± 6.5**	**10.5 ± 2.5**
Chromium	mg/kgdw	**200**	180 ± 70	142.7 ± 29.7
Lead	mg/kgdw	**120**	98 ± 23.1	77.3 ± 10.9
Nickel	mg/kgdw	**110**	66.3 ± 17.6	47.3 ± 6.9
BaP	mg/kgdw	**0.02**	**0.08 ± 0.02**	**0.06 ± 0.01**
Dieldrin	μg/kgdw	**10**	**19.6 ± 16.9**	4.9 ± 2.8

Figure NS6.3. Pollutant levels before and after application of phytomanagement strategies.

The standard (OECD) toxicity bioassays with earthworms -Filter paper, OECD-207 (1984) and reproduction test OECD 222 (2016), shown a great toxicity in the pre-remediation stage. After remediation with phytomanagement strategies, biomass of adult worms and especially reproductive capacity (cocoon umbers and juvenile numbers) significantly improved, the best result being obtained after the triple treatment with P+B+E (Figure NS6.4).

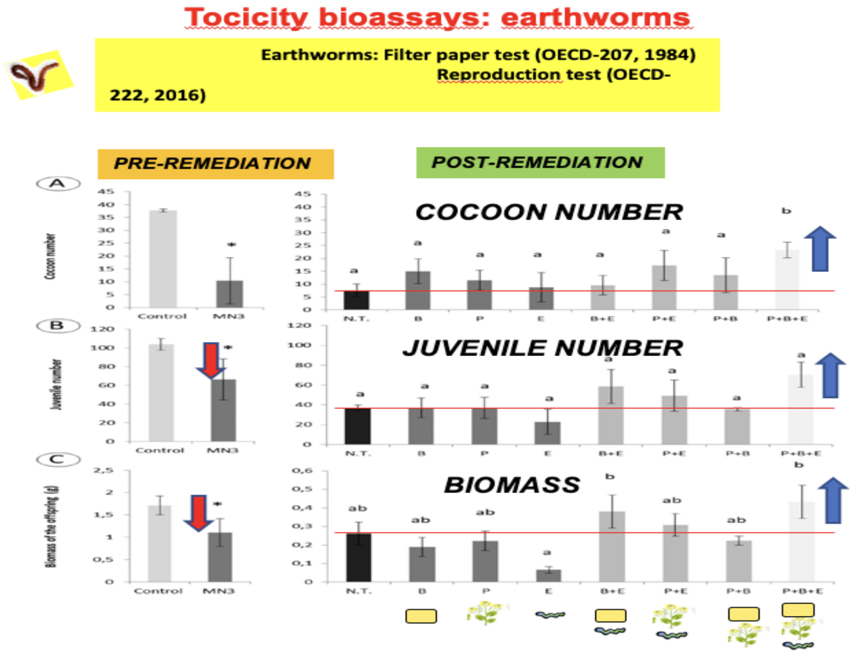


Figure NS6. 4. Toxicity bioassays: Eisenia fetida

Plant phytotoxicity was assessed through germination and root elongation bioassays in three plant species (cucumber, lettuce and onion). Considering the effect on root elongation, cucumber was the most sensitive species to MN3 soil pollution and lettuce the most tolerant (**Figure NS6.5**). Many of the bioassays to estimate phytotoxicity use lettuce as model species, however our results indicate under our experimental conditions that cucumber was the most appropriate species due to its high sensitivity to soil pollution. All applied treatments decrease soil phytotoxicity, but again the best treatment was the combination of the three organisms (P+B+E).

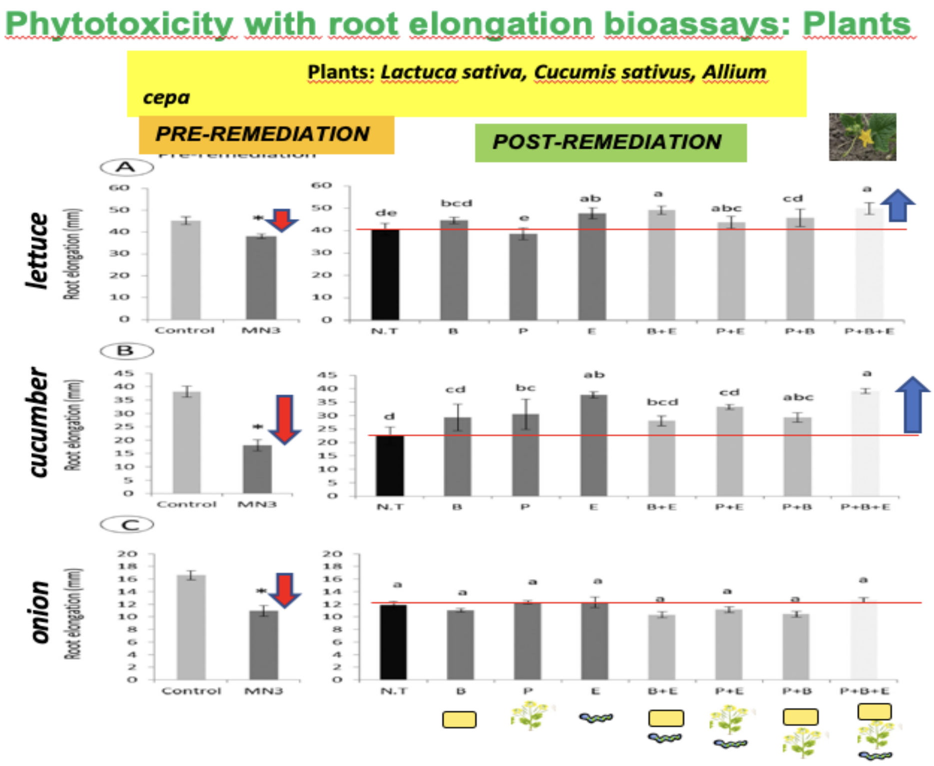


Figure NS6.5. Toxicity bioassays: Phytotoxicity

Soil microbial properties (respiration, biomass and functional biodiversity) were not significantly different among plots (**Figure NS6. 6**). Several factors may explain this effect.

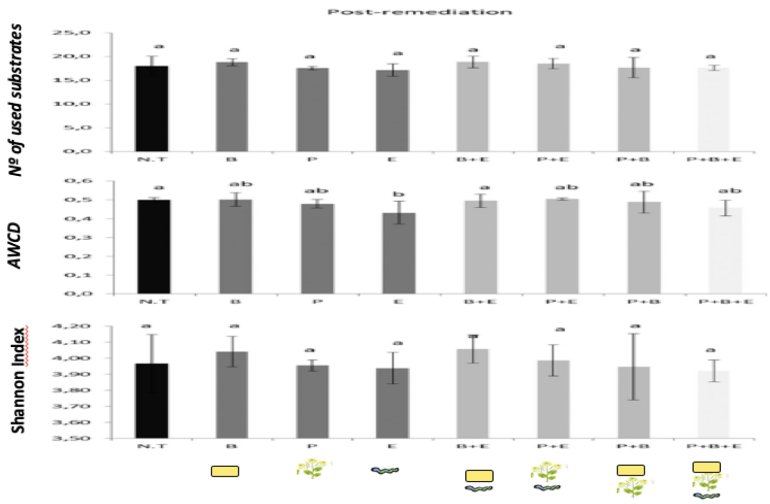


Figure NS6. 6 Toxicity bioassays: Soil microbial populations

The soil was amended with sewage sludge along several years so the level of organic matter was very high in the soil and this can contribute to reduce soil toxicity and bioavailability of pollutants, and in the other hand stimulating bacterial activity and biomass. Besides, contrary to the bioassays with plants and *Eisenia fetida*, both were allochthone's organisms, but in microbial assays we checked the autochthone microbial populations.

Taking all together, the combination of plants (alfalfa)+ worms (Eisenia fetida) + bacterial consortium was the best treatment resulting the best to decrease the levels of pollutants and also to decrease soil ecotoxicity especially for worms and plants in the most polluted plot of the emplacement.

The next strategy was to extend this effective treatment to the entire site except plot MN-8, that was considered a control point (see map in Figure NS6.7).

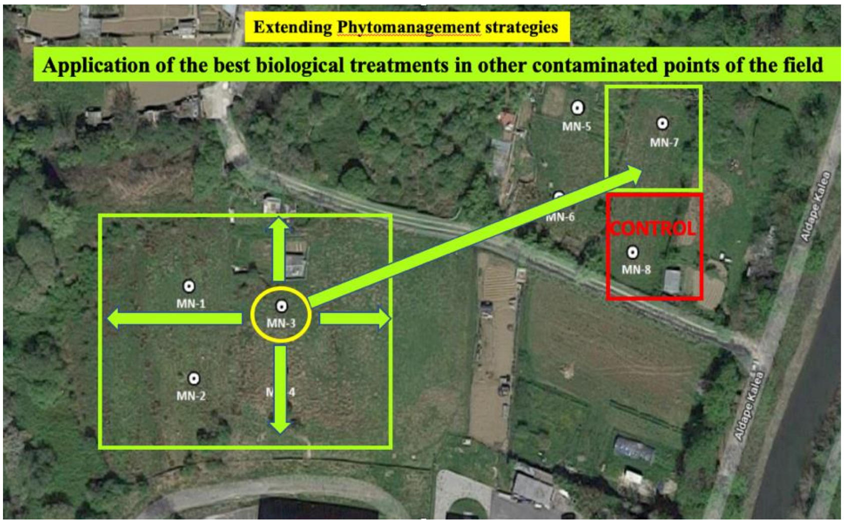


Figure NS6.7. Large scale application of treatment P+B+E

After conditioning the entire site, the best treatment (P+B+E) was applied to the whole area, and the levels of pollutants, vegetation biomass and diversity, phytoextraction rate and bioassays with plants, worms and microbes were carried out. As shown in Figure NS6.8, pollution decreased over time (green background means significantly decreased level of pollutants after phytomanagement treatment.

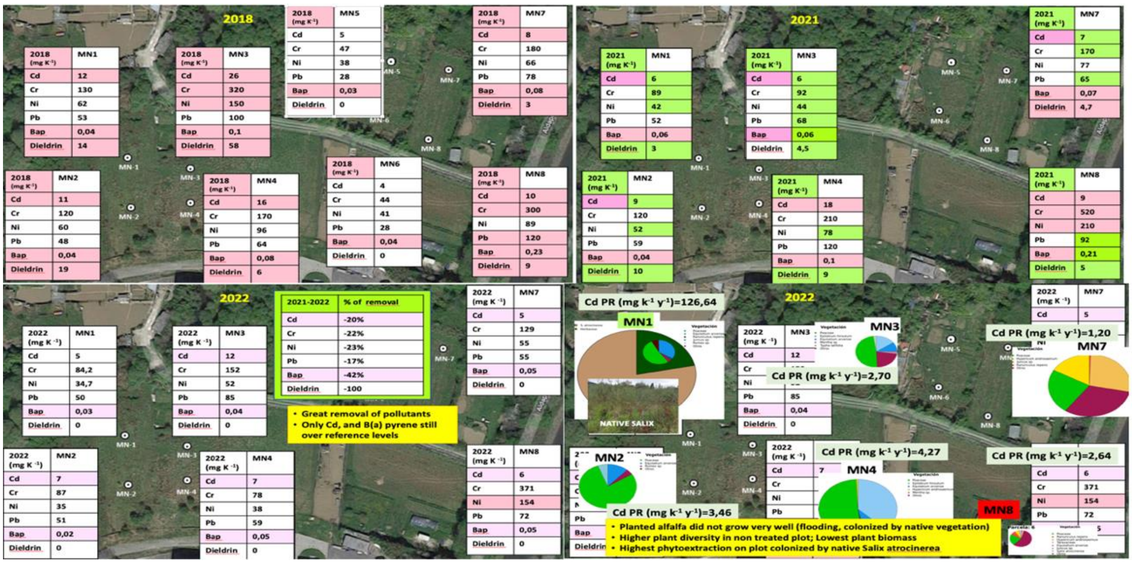


Figure NS6.8. Changes in pollutant concentration after treatment, plant biomass, phytoextraction rate and plant biodiversity

Finally, in 2022 pollutants decreased in all the plots and the dissipation yields were as follows: Dieldrin (100%), Metals (Cd 20%; Ni 23%; Pb 17%; Cr 22%), and Benzo(a)pyrene (42%). As described for the pilot assay, we were able to reduce the levels of metals, Cr, Ni and Pb below the reference levels for contaminated soil, except for Cd. The organic pollutant, dieldrin, was totally degraded, and Benzo(a)pyrene was degraded a 42% although exceeding reference values of polluted soils, and it will requires longer times or for an overall elimination. After one year of phytomanagement, alfalfa plants did not grow very well. In fact, the plots were colonized by flooding tolerant native species with high biomass. The site MN-1 was colonized mainly by *Salix atrocinerea* and this plot had the highest biomass, due to the fast tree growth. Interestingly, this plot presented the highest phytoextraction rate (126,64 mg Kg^{-1}, y-1), while the non-treated plot (MN-8) exhibited the higher levels of pollutants, lowest plant biomass, but the highest plant diversity. Competition among several species and the no intervention in the plot led to a low phytoextraction rate and higher levels of pollutants.

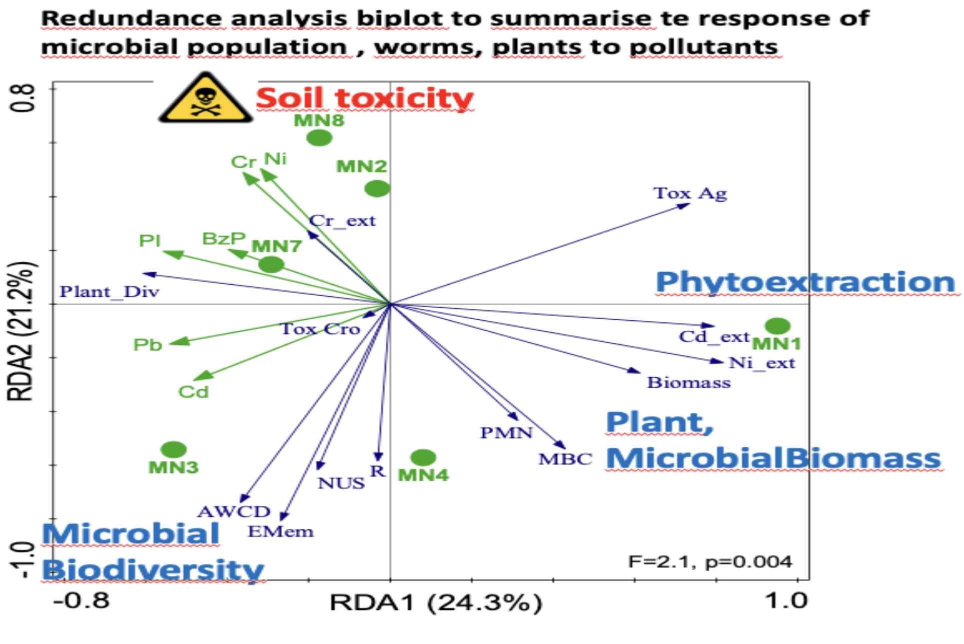


Figure NS6.9. Redundancy analysis biplot for plant, worms and microbial bioassays.

Figure NS6. 9 summarizes soil ecotoxicity and pollutants levels at the end of the phytomanagement strategy. The plots with the highest plant biomass and microbial biomass (MN-1 and MN-4) exhibited also the highest phytoextraction rates and the lowest metal levels. Soil pollution was associated to the non-treated plot MN-8. Other plots with reduced contaminant levels and high plant diversity also showed a high microbial biodiversity.

Conclusion and future use:

Phytomanagement strategies using plants, worms and microbes combined can be a very good strategy to cope with mixed contamination in order to eliminate metals from soil through phytoextraction and at the same time increase organic pollutant degradation. The elimination of some pesticides such as dieldrin can be achieved at medium term but PAHs compounds can take longer times. Decreasing levels of pollutants and the presence of the organism applied greatly improved soil health. One limitation can be that these treatments can be expensive for very large areas and the application of worms should be with an autochthonous ones. According to the owners of the site (Gernika city hall), it is expected that this site can become a park with didactic mission to show schoolchildren and citizens the efficiency of phytomanagement technologies to recover polluted sites.

NS7 Zumanakotxa:

The site is located in the industrial zone of Jundiz (West of Vitoria-Gasteiz), in a place that was originally planned as green area, but due to the abandon and its peripheral location, suffered uncontrolled dumping and illegal spills in the last decades, thus creating a series contaminated plots that degrade the environmental quality and landscape (Figure NS7.1). The anthropic landfills are very variable in typology and depth, consisting mostly of excavation lands and rocks, construction and demolition waste and others industrial or agronomic wastes. Since the area was included in the Basque catalogue of potentially contaminated soils, an analytical study by the city hall administrators was carried out for its chemical characterization.

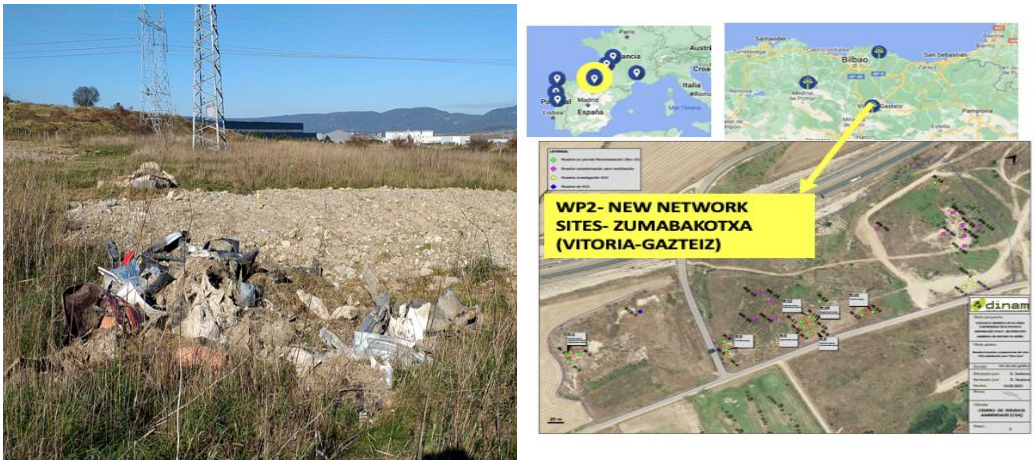


Figure NS7.1. Zumabakotza emplacement in the green belt of Vitoria-Gasteiz

● **Implementation**:
Landfill has been used as a dumping site for construction, demolition, industrial and pesticide residues. As a result the soil was polluted with TPH, PCB, PAH (b/a) pyrene, dibenzene-(a,h)-anthracene and aldrin. In this context this emplacement was included as a new emplacement as part of the Phy2sudoe network to implement Phytomanagement strategies.

This peri-urban site suffered from uncontrolled dumping and illegal spills, thus creating a series of contaminated plots that degrade the environmental quality and landscape. Anthropogenic landfills are very variable in typology and depth, consisting mostly of excavation lands and rocks, but in some cases also include construction and demolition waste and others. Field sampling detected the presence of pollutants at different depths. The phytomanaged plots include areas where the pollutant concentration exceeded the limits set by the regional Basque legislation (VIE-B levels for agricultural use). The compounds detected overpassing reference levels were aliphatic petrol hydrocarbons (TPH), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) and aldrin.

In 2021 CEA hired and directed the installation works of the new phytomanagement plots proposed at Vitoria-Gasteiz (Spain) that included the following phases:
- Preliminary cleaning: due to the state of abandonment and degradation of the plots, it was necessary to remove manually and with machinery numerous debris and garbage items.
- Land preparation: once the area was cleared, earthmoving work began to level several mounds formed by a mixture of materials (earthy, stony, demolition debris). In these operations construction waste (mainly concrete and asphalt agglomerate) was removed, leaving the inert materials of aggregates and earth on the ground to be reused.
- New topography: new contour lines were shaped using the earthy materials, meadows were established over flatter areas, forests over small mounds and lowlands were dedicated to collect runoff water.
- An organic amendment was applied with sewage sludge and shredded pruning (using an approximate dose of 100 t/ha).

Pict. NS7.1. Site cleaning setting aside residues (asphalt) and inert material (@Vilela, CEA)

Pict.NS7.2. Site preparation for poplar/willow stands (@Vilela, CEA)

Fig. NS7.2. Location of plots of Zumababakotza to carry out phytomanagement strategies (each colored star represent a polluted plot).

- **Soil properties**

During 2020 several actions were done to remove manually and with machinery big solid residues (Figure 2). Construction/demolition wastes (mainly concrete and asphalt agglomerate) were removed, leaving the inert materials (rocks and earth) on the ground to be reused. Considering a future use as a park, the area adopted a new topography for landscape integration by moving soils to create flatter areas, small mounds and lowlands to allow runoff and collect water in some places.

The soil had several problems as poor structure, low content of organic matter and nutrients and quite high pH; so, in 2021, prior to plantation an organic amendment (sewage sludge plus shredded pruning) was applied using an approximate dose of 100 t/ha. Besides soil characterization indicated the presence of several organic and inorganic pollutants.

Soil pollution analysis (after earthworks, before planting):

In November 2020, CEA performed a time zero (t0) contamination analysis where each treatment was to be applied, running a soil analysis on subplots as follows:

NS7a: control. M15. 1 analysis

NS7b: Holm oak forest. M13, M14. 2 analyses

NS7c: restoration crop (alfalfa + ryegrass). M8, M10, M12. 3 analyses

NS7d: gall oak forest. M7, M9, M11. 3 analyses.

NS7e: poplar/willow/alder on mulch. M4, M5, M6. 3 analyses

NS7f: scrub. M1, M2, M3. 3 analyses

CEA evaluates changes in pollutant linkages on the site associated with the phytomanagement options, especially CEA is monitoring the contamination reduction on each treatment.

Figure NS7.3. Initial chemical characterization of organic pollutants (2001): concentration and location.

Pic. NS7.7.3. NS7 plots and monitoring subplots (@Vilela, CEA)

After the chemical analysis of soils (Figure NS7.3), three relevant aspects were deduced: (i) only the organic pollutants (TPH, PCB, PAH (b/a) pyrene, dibenzene-(a,h)-anthracene and aldrin) surpassed reference levels for contaminated soils (VIE-B); (ii) the distribution of these compounds was very heterogeneous, so each plot had different organic pollutants; and (iii) these compounds were at low-medium concentration. In more detail, the concentration of pollutants in the selected polluted plots was as follows (in mg/kg): S7c: PCB 0,011-0,016, b(a)pryrene 0,0211-0,0669 and dibenzene (a,h) anthracene 0,04 ; S7d: b(a) pryrene 0,481; S7e: TPH 53; S7f: TPH 100, aldrin 0.0119;and b(a)pryrene 0,02. Soil qualitative analysis with soil cards was also performed.

In situ assessment of soil quality with Soil Cards at the Zumabakotxa site © Neiker/ CEA

● **Phytomanagement options / plant assembly**

- From autumn of 2021 to spring 2022, sowing and planting took place, placing one type of plant community to each phytomanagement plot, thus obtaining various treatments and potential trajectories for new (socio)ecosystems as follows:

NS7a: control

NS7b: holm oak forest

NS7c: restoring crop (alfalfa + ryegrass)

NS7d: gall oak forest

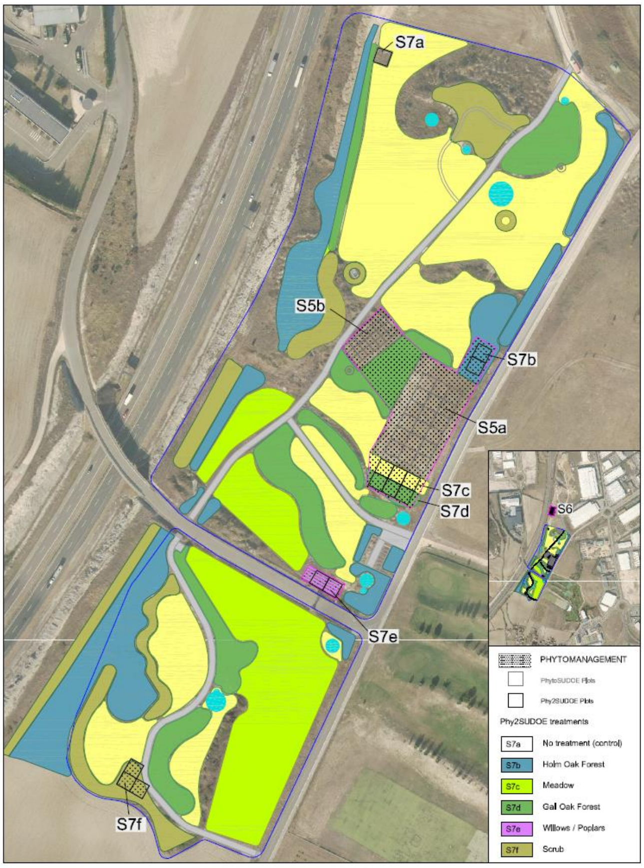
NS7e: poplar/willow/alder stands

NS7f: scrubland

For a complete list of plant species find the link below in the section Documents.

- A network of roads and irrigation lines was established to facilitate later maintenance work.

- In November 2022 dead plantings were replaced



Pic. NS7.7.2. Site planting plan and Phy2Sudoe plots (@Vilela, CEA)

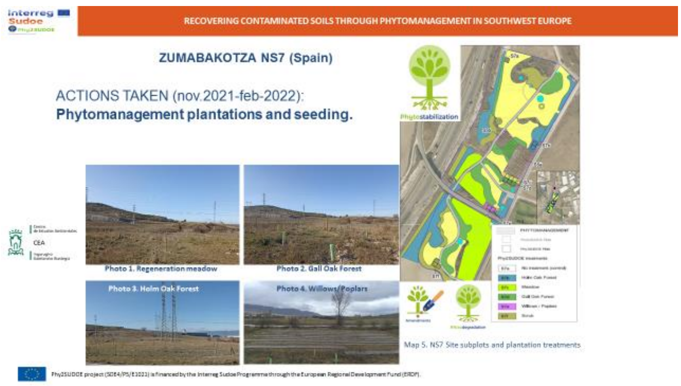


Fig. NS7. 7.3 Map and phytomanagement set-up (@Vilela, CEA)

Considering the edapho-climatic characteristics of each plot, the level and kind of pollutants, the future land use for the site and the potential vegetation of the area an "ad hoc" vegetation were selected for each polluted plot. In more detail, the plant species used in each plot were as follows: S7c: Meadow (alfalfa + ryegrass); S7d: gall oak; S7e: poplar / willow/ alnus; S7f: several shrub Mediterranean species (Figure NS7.4).

● **Success / limits**

CEA is maintaining the plots (replanting of died plants, weeding, etc.) and doing the soil and plant analysis in line with Neiker and UPV. Soil qualitative analysis were done with Soil Cards in April 2022 (CEA and Neiker). Changes in soil pollution is regularly under investigation, as well as plant analysis.
Globally phytomanagement options are successful. The key-point is the water supply (so the irrigation network was relevant) and tolerance to heatwaves and drought.
Pollution analysis (after planting):
In June 17th 2022, sometime after planting, the pollution analysis was repeated, contrasting the values with the regional reference levels (VIE-B). All values showed a decreased and were below the limits for public park use, which was the project objective.
Results of pollution control are summarized in the following table

Parcela	Muestra	Parámetro	Unidad	otros	2015	2020	2022
NS7a	M16	PCBs	mg/kgms	0,01	0,12	<0,01	<0,01
NS7a	M16	Benzo(a) pireno	mg/kgms	0,02	<0,02	<0,02	0,0343
NS7b	M14	Benzo(a) antraceno	mg/kgms	0,2	0,95	<0,2	<0,2
NS7b	M14	Benzo(b) fluoranteno	mg/kgms	0,2	0,96	<0,2	<0,2
NS7b	M14	Benzo(a) pireno	mg/kgms	0,02	0,66	<0,02	<0,02
NS7b	M14	Dibenzeno(a,h) antraceno	mg/kgms	0,03	0,06	<0,03	<0,03
NS7b	M14	Indeno(1,2,3-cd) pireno	mg/kgms	0,3	0,44	<0,3	<0,3
NS7b	M14	Hidrocarburos totales C5-C40	mg/kgms	50	<25	57,4	<25
NS7c	M12	Benzo(a) pireno	mg/kgms	0,02	0,06	0,0211	<0,02
NS7c	M12	Suma 7 PCB (BallSmiter)	mg/kgms	0,01	<0,01	0,011	<0,01
NS7c	M8	Benzo(a) pireno	mg/kgms	0,02	<0,02	0,0669	<0,02
NS7c	M8	Suma 7 PCB (BallSmiter)	mg/kgms	0,01	<0,01	0,016	<0,01
NS7c	M10	Benzo(a) pireno	mg/kgms	0,02	<0,02	0,131	<0,02
NS7c	M10	Suma 7 PCB (BallSmiter)	mg/kgms	0,01	<0,01	0,0452	<0,01
NS7d	M7	Benzo(a) pireno	mg/kgms	0,02	0,02	<0,02	0,0226
NS7d	M7	PCBs	mg/kgms	0,01	0,041	<0,01	<0,01
NS7d	M7	Suma 7 PCB (BallSmiter)	mg/kgms	0,01	<0,01	<0,01	0,0123
NS7d	M9	Benzo(a) pireno	mg/kgms	0,02	<0,02	0,0481	<0,02
NS7d	M11	Benzo(a) pireno	mg/kgms	0,02	<0,02	<0,02	0,0432
NS7e	M6	PCBs	mg/kgms	0,01	1,4	<0,01	<0,01
NS7e	M6	Acetona	mg/kgms	1	4	<1	<1
NS7e	M5	Suma 7 PCB (BallSmiter)	mg/kgms	0,01	<0,01	<0,01	<0,02
NS7e	M5	Hidrocarburos totales C5-C10	mg/kgms	50	<25	53,9	<25
NS7f	M1	Benzo(b) fluoranteno	mg/kgms	0,2	0,27	<0,2	<0,2
NS7f	M1	Benzo(a) pireno	mg/kgms	0,02	0,22	<0,02	<0,02
NS7f	M1	Dibenzeno(a,h) antraceno	mg/kgms	0,03	0,05	<0,3	<0,3
NS7f	M1	Dieldrin	mg/kgms	0,01	0,021	<0,01	<0,01
NS7f	M1	Hidrocarburos totales C10-C40	mg/kgms	50	100	<25	<25
NS7f	M2	Aldrina	mg/kgms	0,01	<0,01	0,0119	<0,01
NS7f	M2	Benzo(a) pireno	mg/kgms	0,02	<0,02	0,0207	<0,02
NS7f	M2	Hidrocarburos totales C5-C10	mg/kgms	50	<25	100	<25
	Valores que superan VIE-B "Otros usos"						

Pic.7. 3. Pollution analysis results in 2015, before planting (2020) and after planting (2022)

At the end of spring 2022, a chemical analysis to assess potential pollutant degradation was done. Main results indicated that the levels of organic pollutants decreased in all plots (**Figure NS7.4**). In fact, on S7f and S7c the levels were similar to those of uncontaminated soils. Only S7e and S7d had PBC and b(a)pryrene, respectively, surpassing the regulatory limits, but very close to these limits. At the rates of degradation observed in the 8 months of our experiment it would take a few months more to decrease the level of these pollutants under regulatory limits. It should consider that the regulatory limits considered until now have been the most restrictive ones (agricultural use). Considering that the future use of the site will be a park all the plots are now under regulatory limits and the soil should considered as a non-polluted for park use.

From 2021 to 2022 soil qualitative analysis with soil cards were done. As shown in Figure 5 soil parameters determined were low, typical values for a degraded soil.

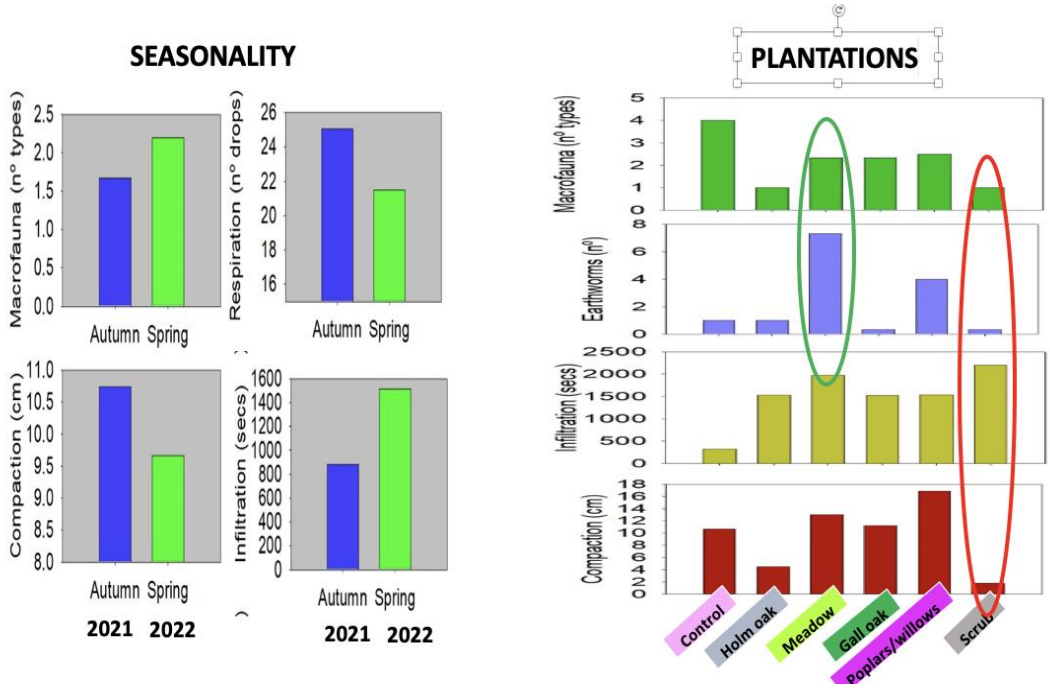


Figure NS7. 5. Soil qualitative analysis with soil cards.

These values were affected by seasonality, reaching the better values of macrofauna biodiversity, microbial respiration, and very low compaction and infiltration in spring. The implanted vegetation could also play a relevant role since at the end of the study the best indicators were found in the "Meadow" treatment planted with alfalfa and ryegrass. However, plantations with Mediterranean bushes (Schrub) presented the worst indicators of soil health status. It is important to highlight that these parameters have been carried out as part of a **"citizen science program"** to make citizens aware of the importance of soil and bring science closer to their daily lives. An important objective of the project is to disseminate scientific knowledge and involves other stakeholders.

In July 2022, a study was carried out to determine performance of the planted species in each plot. Thus, biometric parameters (**Figure 6A**), and physiological status and content of photosynthetic pigments and antioxidants (**Figure 6B**) were analyzed.

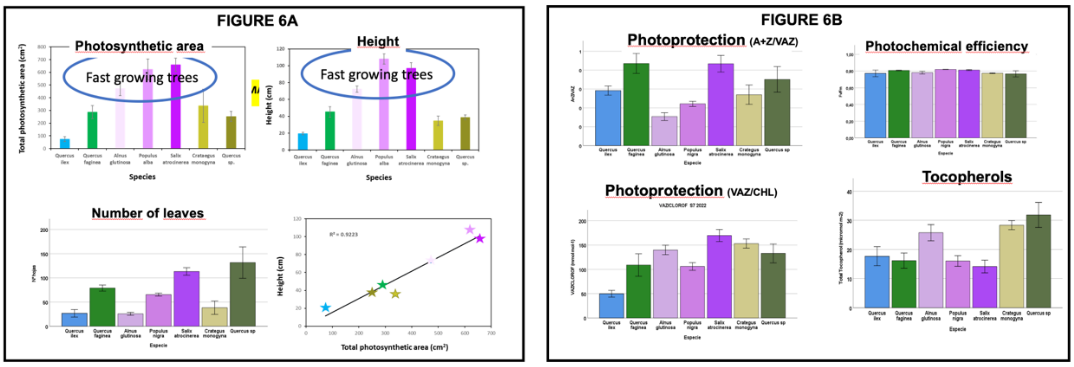


Figure 6. Plant performance: (A) Biometric parameters and (B) physiological parameters and content of photosynthetic pigments and tocopherols.

Although during the maintenance the plots were irrigated, due to the drought suffered that year, some of the trees died and latter have been replaced. In any case, the data presented correspond to the trees that survived on the field. As expected, the highest growth (height, total photosynthetic area) occurred in alders, poplars and willows, although the growth of the other species was also quite good (**Figure 6A**). An interesting fact was that despite the very unequal number of leaves among the tree species, the photosynthetic area correlated with the height of the trees. So, at this stage height can be used as a proxy of total photosynthetic area. The physiological parameters indicated a good health status of the trees, with values photochemical efficiency near to 0,8 for all species (**Figure 6B**). The trees of the genus Quercus and Crataegus showed the highest level of photoprotection and antioxidant content that provide them a greater tolerance to stress compared to fast-growing species. These data indicate that special care must be taken in these early stages with willow, poplar and alder species to avoid, above all, water stress that compromises their survival, since their physiological capacity to overcome stress is lower than of the other Mediterranean species.

Data included total photosynthetic area, leaf area index, maximum shoot length, DW yield of plant parts, photosynthetic efficiency, chlorophyll, carotenoids, and tocopherols.

Qualitative soil analysis (NEIKER): is ongoing Qualitative soil analysis (soil cards)

Field trials at Zumabakotxa, Spain (© J Vilela et al., CEA)

Conclusion and future use:

Polluted peri-urban areas whose future could be to become parks can be rehabilitated using phytomanagement strategies and landscape criteria. The selection of the most appropriate plant species (herbaceous and trees) for each ecological niche identify in the area can guarantee long-term survival of plants, low maintenance, greater integration with the surrounding area, increase ecological biodiversity and sustainability. On the other hand, an organized plan for the preparation of the land, the addition of organic amendments and the implantation of vegetation “ad hoc” can operate a progressive decontamination of organic compounds, feasible when these levels are not very high, allowing new uses for these soils improving ecological, economic and social values of the area.

NS8 - Estarreja, Portugal:

● **Implementation**

This site in the vicinity of a large chemical industrial complex is an area (close to Estarreja, a small town with a population of around 7800 inhabitants, located in central Portugal, 14.5 km northeast of Aveiro) with high permeability and an average depth to groundwater level of less than 1 meter, with seasonal flooding due to the aquifer rise. This led to build a network of drainage ditches, which allowed the agricultural land use. Over 5 decades, from about 1940 onwards, the chemical complex used some ditches for the discharge of wastewater with high content of aniline and derivatives, other organics, ammonia and metals(loid)s, mainly As, Hg, Pb and Zn. In the vicinity of the experimental site, where some lower areas naturally become shallow ponds for a third of a year, the ditch designated as “Vala de S. Filipe” was frequently submerged, facilitating the dispersion of the industrial wastewater discharges, sedimentation and contaminant transfer to the groundwater.

High levels of Hg and As posing unacceptable risks to human receptors have led to a Risk-based Remediation Project that, within that area, required the excavation of the topsoil (to a general depth of 25 cm and, in some ‘hotspots’, to a minimum depth of 50 cm), to be replaced by uncontaminated soils. The new soils, however, are expected to be impacted by the seasonal rise of the residually contaminated groundwater.

● **Geological, geomorphological and hydrological framework**

The study area fits into the larger tectono-stratigraphic zones defined by Ribeiro et al. (1979) in the Western Mesocenozoic Rim, which corresponds to an extensive sedimentary filling that was deposited in an elongated trench NNE-SSW, resulting from the opening and subsequent spreading of the Atlantic Ocean (Lusitanic Basin), whose sediments were later remobilized by a tectonic activity compressive.

In the area of the sedimentary basin of Aveiro, which encompasses the site where the work is carried out, the sediments and base are from the lower Cretaceous and are based on discordance over the ancient soils of the Pre-Cambrian schist-grauvachic basement. The sedimentary basin of Aveiro is characterized by very flat reliefs, with elevations close to hydrographic zero along the coast and lower than (100) on its eastern edge. According to the Geological Map of Portugal, 1/50,000, sheet 13-C Ovar, detrital sedimentary deposits of Holocene age appear. These deposits based discordantly on a substrate constituted by clayey-grey formations from the Lower Cretaceous, which based discordantly on Precambrian schists.

The Holocene deposits correspond essentially to dune sand, beach sand and alluvium. Dune and beach sand are fine to very fine sand, with a weak clay component. The alluvium, associated with the main rivers and other water lines of lesser expression, are sandy-mud and silt-mud.

The Quaternary Aquifer System is composed of 2 aquifer units. (Ordens, 2007):

• First aquifer unit: this is a superficial phreatic aquifer and lithologically it is installed, from top to bottom, in surface sand (dune), fine sand sometimes with small pebbles, intercalations of silt and muddy sand with vegetable remains. This unit has high permeability and porosity, which makes it very vulnerable to pollution. Its recharge is carried out through atmospheric precipitation.

• Second aquifer unit (known as “Quaternary Base”): this is a semi-confined aquifer lithologically installed, from top to bottom, in fine sand to coarse sand with round pebbles and medium to coarse sand with round pebbles. It features intercalations of silt and muddy sand with plant remains. Its recharge basically depends on the infiltration of water from atmospheric precipitation.

● **Site implementation**

The Phy2Sudoe NS8 site is located west of the Estarreja Chemical Complex in a non-intervention area adjacent to the remediated area. Following the phytoremediation layout by UCP, a total of 20 discrete soil samples were collected along 20 lines (1 point per line), in a zig-zag scheme. The location of the sampling points has been geo-referenced using a Leica Geosystems reference station.

Delays in the local large remediation project have delayed the deployment of the experimental site, thus limited the period for experimental phytoremediation.

Sampling was carried out in two periods, in July 2022 and March 2023.

Samples were collected 0 to 15 cm deep, using a stainless-steel shovel and samples were stored in plastic bags and sent to an accredited laboratory to be analyzed for the following parameters: Dry matter Total Organic Carbon (TOC), Arsenic, Barium, Beryllium, Calcium oxide(CaO), Cadmium, Cobalt, Chromium , Copper, Mercury, Potassium oxide (K_2O), Magnesium (as MgO), Molybdenum, Sodium (as Na_2O) Nickel, total Phosphorus (as PO_4), Lead, Sulphur; Sulphur, Antimony, Selenium, Tin, Vanadium, Zinc, Acidity (pH-H_20), mono-aromatic hydrocarbons, (aliphatic and aromatic fractions), polycyclic aromatic hydrocarbons (PAH), total hydrocarbons (THP) and Kjeldahl Nitrogen.

The ISO/IEC-17025:2005-accredited analytical laboratory that was subcontracted (Eurofins NL, Barneveld, with the Accreditation Certificate L010 by the Dutch Accreditation Council RvA) has used the following techniques, according to standard methods and internal procedures that are regularly audited:

- pH: potentiometry;
- Conductivity: electrometry;
- Elements ICP/MS;
- Dry matter: thermogravimetry;
- TPH, PAH, BTEX – GC/MS;
- TOC: Dry Combustion
- Anions: IC
- Phosphorus: VIS
- Kjeldahl Nitrogen: Persulphate oxidation/FIA-VIS

To evaluate the spatial distribution of contaminants, descriptive statistical and geostatistical analysis were performed using the SADA — Spatial Analysis and Decision Assistance software (version 5.0), by the Oak Ridge National Laboratory and the University of Tennessee.

Different models of semi-variograms were established for the analyzed parameters, to establish the most appropriate geostatistical method.

The correlations between variables were assessed by a Pearson correlation analysis, providing an effective way to highlight the relationships between multiple variables and to support the understanding of influencing factors.

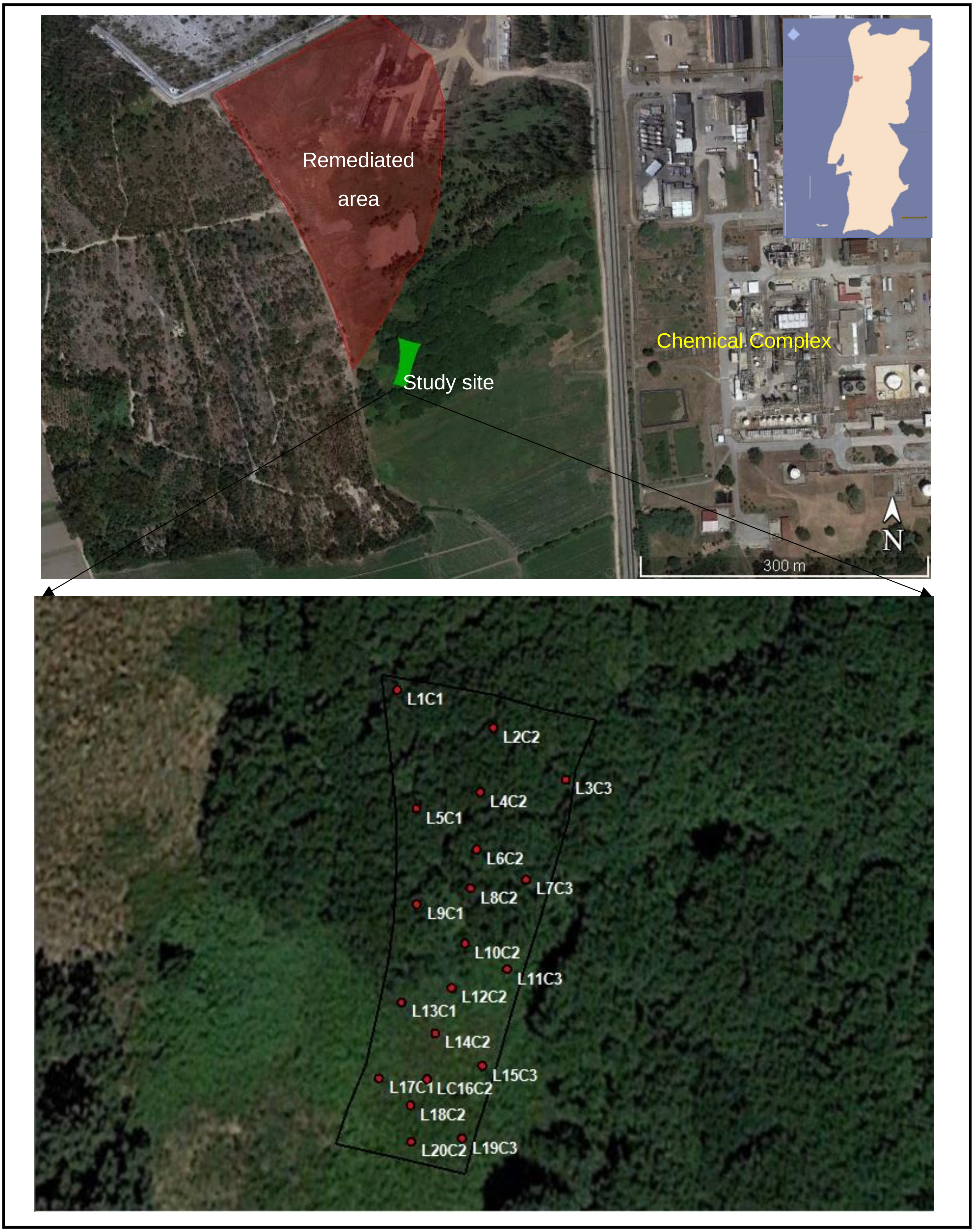
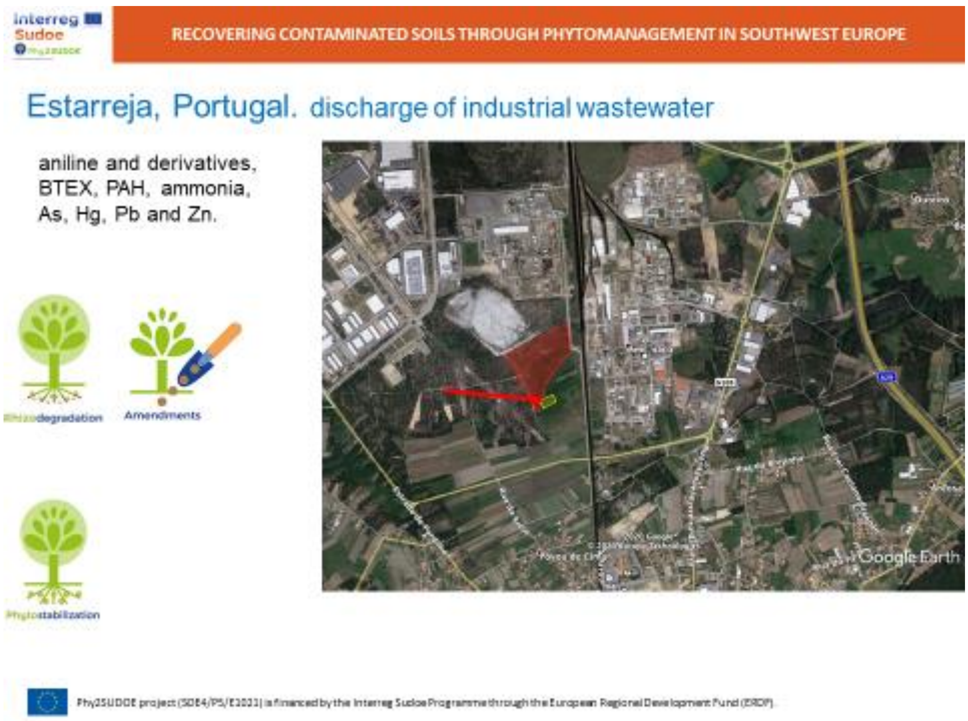


Figure NS8.1. Estarreja site

- **Soil properties**

Main contaminants before remediation average 3298 mg As/kg (about 3000 mg/kg at 25 cm depth) and 89 mg Hg/kg (about 50 mg/kg at 25 cm depth). Organic contaminants have not been quantified, but due to the site history, hydrocarbon contamination is known to be present: benzene, toluene, ethylbenzene, xylene, and PAH.

Sampling of soil and soil macro-fauna using Pitfall traps were carried out. 14 sampling points were assessed in the study area and 1 in the reference area. 16 bait lamina were deployed at each soil sampling point and 20 in the reference sampling point. The bait-lamina test is an in situ method intended to evaluate the feeding activity of soil organisms.



Assessment of physico-chemical and biological properties at the Estarreja site © UCP

Aniline and its derivatives, BTEX, PAH, ammonia, As, Hg, Pb and Zn were present in excess on the site

Several tasks are ongoing: phytoremediation planning according to the analytical characterization of the site, phytoremediation deployment, retrieval and analysis of bait lamina, and processing of the collected samples of macro fauna.

Table NS8.1 shows the analytical results of both samplings and table NS8.2 shows the exploratory data analysis results. Considering an agricultural occupation of the land with groundwater abstraction for irrigation purposes, Arsenic (mean: 42.40 mg/kg in 1st sampling) and (mean:33.12 mg/kg in the 2nd sampling), Mercury (mean: 5.38 mg/kg in 1st sampling) and (mean:3.38mg/kg in the 2nd sampling), and Lead (77.50 mg/kg in 1st sampling and (mean: 58.20 mg/kg in the 2nd sampling) exceed the national reference values for many of the sampling points. These 3 elements were found to be highly variable, ranging between 11 mg/kg and 190 mg/kg in 1st sampling and ranging between 6.4 mg/kg and 88 mg/kg in the 2nd sampling for Arsenic; 0.29 mg/kg and 23 mg/kg in 1st sampling and ranging between 0.23 mg/kg and 14 mg/kg in the 2nd sampling for Mercury; 20 mg/kg and 340 mg/kg in 1st sampling and ranging between 12 mg/kg and 210 mg/kg in the 2nd sampling for Lead. These results show the significant spatial variability occurring in this site. Regarding metal(loid)s, Molybdenum and Tin were not detected and Cadmium and Nickel were detected in less than 3 samples. Those 4 elements were excluded in the statistical analysis. No mono-aromatic hydrocarbons were detected. Aliphatics >C21 - C35 were detected in 2 sampling points (L5C1 and L9C1) in the 1st sampling and Aliphatics >C10 – C12 were detected in 5 sampling points (L8C2; L10C2; 11C3; L12C2 and L13C1) in the 2nd sampling.

Polycyclic Aromatic Hydrocarbons were detected in 5 points (L1C1; L9C1; L13C1; L15C3; and L17C1). All detected concentrations were very low. Benz(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Chrysene, Fluoranthene, Indeno(1,2,3-cd)pyrene, PAH 10 VROM (sum), PAH 16 EPA (sum), Phenanthrene and Pyrene were detected in the 1st sampling and Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Chrysene, Fluoranthene, Indeno(1,2,3-cd)pyrene and Pyrene were detected in in the 2nd sampling .

Pearson's correlation (table NS8.3 and table NS8.4) has highlighted a positive correlation between several metal(loid)s. Arsenic strongly positively correlates with Ba (0.98 and 0.92), CaO (0.95 and 0.91), Hg (0.93 and 0.92), Pb (0.99 and), and pH (0.85 and 0.75) ; Mercury has a strong positive correlation with As (0.93 and 0.92), Ba (0.92 and 0.93) CaO (0.90 and 0.93), Pb (0.92 and 0.94) and pH (0.91 and 0.86); Lead strongly positively correlates with As (0.99 and 0.97), Ba (0.98 and 0.94), CaO (0.94 and 0.95), Hg (0.92 and 0.94), and pH (0.86 and 0.86). Calcium, which has a positive correlation with metal(loid)s, is the element with the higher variability.

Table NS8.1 - Chemical analysis of both soil sample series. White line -1st sampling and Blue line – 2nd sampling. Bold numbers represent values above the limit acoordindg (1)- Contaminated Soils – Technical Guide | Soil Reference Values (Table E) considering agricultural occupation with the use of groundwater, according to APA, the Portuguese Environmental Agency.

	Unit	**L1C1**	**L2C2**	**L3C3**	**L4C2**	**L5C1**	**L6C2**	**L7C3**	**L8C2**	**L9C1**	**L10C2**	**L11C3**	**L12C2**	**L13C1**	**L14C2**	**L15C3**	**LC16C2**	**L17C1**	**L18C2**	**L19C3**	**L20C2**	**RV[1]**
Characteristics																						
Dry matter	% (w/w)	96.8	92.5	91.5	92.8	95.2	93.5	94.2	93.7	90.3	92.0	94.5	91.5	93.7	93.0	94.9	90.9	91.4	91.3	94.1	91.9	**na**
		71.5	78.3	76.0	78.0	82.2	83.1	80.1	86.7	85.4	82.7	83.7	84.9	84.6	89.1	83	83.4	86.8	82.4	82.9	84.7	
Total Organic Carbon (TOC)	g/kg ms	17	14	16	17	11	18	12	11	21	19	13	14	21	16	14	18	14	13	12	13	**na**
		26	20	22	16	20	21	15	14	22	16	10	11	17	12	13	11	15	15	11	8.6	
Elements																						
Antimony (Sb)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.1	2.4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	**7.5**
		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	4.8	<2.0	<2.0	2.8	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	
Arsenic (As)	mg/kg dm	**55**	**38**	**30**	**66**	**51**	**46**	**190**	**18**	**41**	**63**	**79**	**11**	**25**	**23**	**17**	**31**	**18**	**17**	**11**	**18**	**11**
		39	**27**	**22**	**82**	**53**	**33**	**67**	**27**	**48**	**88**	**28**	**13**	17	6.4	24	26	19	16	13	14	
Barium (Ba)	mg/kg dm	92	59	28	76	78	61	340	22	46	87	100	<15	31	29	21	35	21	24	17	27	**390**
		73	47	25	74	56	43	74	62	50	120	33	18	17	<15	28	27	32	24	18	21	
Calcium oxide (CaO)	mg/kg dm	4000	1500	2100	3300	3300	61	12000	1800	<50	5000	5100	520	1400	1100	800	2800	570	880	640	910	**na**
		1900	990	810	2500	1900	1600	3700	1500	1800	7200	3000	550	550	350	740	670	600	630	480	500	
Chromium (Cr)	mg/kg dm	<5.0	5.5	<5.0	5.9	7.0	5.2	<5.0	<5.0	<5.0	<5.0	6.0	<5.0	5.3	6.3	5.2	8.4	<5.0	6.6	5.9	6.0	**160**
		5.8	5.4	<5.0	5.7	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	5.7	5.9	5.3	6.2	5.1	5.1	
Copper (Cu)	mg/kg dm	13	12	10	12	15	12	21	5.7	15	16	22	9.3	14	15	14	22	15	16	12	16	**180**
		15	12	11	16	14	12	15	9.3	15	18	17	13	14	5.6	15	17	15	18	13	15	
Lead (Pb)	mg/kg dm	**95**	**66**	40	**130**	**110**	**79**	**340**	30	**65**	**130**	**160**	20	41	**48**	27	**53**	30	32	22	32	**45**
		66	**46**	32	**100**	**97**	**68**	**120**	**60**	**70**	**210**	**54**	27	28	12	35	38	30	29	21	21	
Magnesium as MgO	g/kg dm	870	1100	970	980	1000	1500	700	730	860	630	910	520	1000	1200	960	1600	760	1200	1100	1100	**na**
		1300	1100	1100	1300	990	850	750	540	770	710	710	700	810	480	960	870	1300	1200	1100	1100	
Mercury (Hg)	mg/kg dm	**22**	**3.1**	**2.2**	**6.3**	**10**	**4.0**	**23**	**1.9**	**6.4**	**8.9**	**9.9**	0.56	**2.7**	**1.6**	0.71	**1.2**	**1.2**	0.73	0.29	1.0	**1.8**
		8.9	**1.8**	1.2	**6.2**	**6.7**	**3.5**	**7.7**	**2.5**	**6.7**	**14**	**3.2**	0.57	0.74	0.35	0.93	0.76	0.86	0.52	0.23	0.32	
Phosphorus	mg/kg dm	0.14	0.17	0.16	0.15	0.17	<0.050	0.16	0.087	<0.050	0.13	0.23	0.13	0.19	0.24	0.14	0.25	0.14	0.16	0.12	0.14	**na**

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

	Unit	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2	RV[1]
		0.19	0.19	0.15	0.21	0.13	0.16	0.17	0.11	0.16	0.16	0.13	0.17	0.18	0.1	0.17	0.18	0.18	0.18	0.14	0.14	
Potassium oxide (K_2O)	g/kg dm	0.46	0.59	0.50	0.47	0.52	0.69	0.36	0.24	0.44	0.33	0.44	0.29	0.55	0.55	0.38	0.63	0.37	0.53	0.50	0.51	**na**
		0.69	0.60	0.55	0.69	0.51	0.49	0.4	0.29	0.42	0.33	0.37	0.39	0.46	0.27	0.46	0.42	0.7	0.52	0.51	0.54	
Selenium (Se)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	**4.7**	<2.0	<2.0	2.1	2.2	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	**2.4**
		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	**2.9**	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	
Sodium (Na_2O)	mg/kg dm	110	160	140	140	150	<50	100	52	<50	68	100	150	100	110	65	150	56	100	150	94	**na**
		200	220	230	140	71	180	220	120	210	170	140	140	140	130	200	180	230	160	220	180	
Sulphur (S)	g/kg dm	0.22	0.26	0.28	0.27	<0.20	<0.20	0.41	<0.20	<0.20	0.22	0.35	<0.20	0.41	0.36	0.26	0.40	<0.20	0.22	0.22	0.36	**na**
		0.33	0.29	<0.20	0.25	<0.20	<0.20	0.3	<0.20	0.26	<0.20	0.22	<0.20	<0.20	<0.20	0.23	0.2	<0.20	<0.20	<0.20	<0.20	
Sulphur as sulphate (SO_4)	g/kg dm	0.66	0.77	0.85	0.82	<0.60	<0.60	1.2	<0.60	<0.60	0.67	1.0	<0.60	1.2	1.1	0.78	1.2	<0.60	0.66	0.66	1.1	**na**
		1.00	0.88	<0.60	0.75	<0.60	<0.60	0.89	<0.60	0.78	<0.60	0.67	<0.60	<0.60	<0.60	0.68	0.6	<0.60	<0.60	<0.60	<0.60	
Vanadium (V)	mg/kg dm	6.2	8.2	6.8	6.9	7.7	8.9	5.5	<5.0	8.1	5.7	7.8	<5.0	8.5	10	6.9	11	6.0	8.2	6.7	7.6	**86**
		9.3	9.8	8.0	9.4	7.2	6.6	6.6	5	7.4	5.8	6.4	6.7	7.9	<5.0	8.3	11	8.8	7.8	7.1	6.6	
Zinc (Zn)	mg/kg dm	81	59	47	72	95	73	140	32	75	86	160	52	82	110	97	140	73	110	92	95	**340**
		80	67	51	95	83	66	84	58	72	110	120	78	73	38	110	110	84	110	99	84	
TPH Aliphatic Aromatic split																						
Aliphatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	**na**
		<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.8	<2.0	3.1	2.6	3	2.3	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	
Aliphatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	14	<12	<12	<12	17	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	**na**
		<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	
Polycyclic Aromatic Hydrocarbons, PAH																						
Benz(a)anthracene	mg/kg dm	0.031	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010	0.016	<0.010	0.012	<0.010	<0.010	<0.010	<0.010	<0.010	**0.63**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	
Benzo(a)pyrene	mg/kg dm	0.029	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.024	<0.010	<0.010	<0.010	0.016	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**0.078**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.016	<0.010	
Benzo(b)fluoranthene	mg/kg dm	0.034	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.032	<0.010	<0.010	<0.010	0.021	<0.010	0.010	<0.010	0.013	<0.010	<0.010	<0.010	**0.78**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.01	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	0.02	<0.010	

	Unit	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2	RV[1]
Benzo(g,h,i)perylene	mg/kg dm	0.021	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.021	<0.010	<0.010	<0.010	0.013	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**7.8**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	
Benzo(k)fluoranthene	mg/kg dm	0.014	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**0.78**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	
Chrysene	mg/kg dm	0.031	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.025	<0.010	<0.010	<0.010	0.018	<0.010	0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**7.8**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.016		
Fluoranthene	mg/kg dm	0.066	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.042	<0.010	<0.010	<0.010	0.027	<0.010	0.014	<0.010	0.011	<0.010	<0.010	<0.010	**0.69**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.012	<0.010	
Indeno(1,2,3-cd)pyrene	mg/kg dm	0.016	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**0.48**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.013	<0.010	
PAH 10 VROM (sum)	mg/kg dm	0.25	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.17	<0.10	<0.10	<0.10	0.11	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	**na**
		<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	
PAH 16 EPA (sum)	mg/kg dm	0.34	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	0.24	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	**na**
		<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	
Phenanthrene	mg/kg dm	0.043	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	0.019	<0.010	<0.010	<0.010	<0.010	<0.010	**7.8**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	
Pyrene	mg/kg dm	0.056	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.036	<0.010	<0.010	<0.010	0.023	<0.010	0.010	<0.010	<0.010	<0.010	<0.010	<0.010	**78**
		<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	
Physical and chemical analyses																						
Acidity (pH-H20)		7.4	6.1	5.6	7.2	6.9	6.8	7.8	6.7	6.1	7.6	7.4	5.3	5.6	5.3	5.4	5.2	5.4	5.2	5.3	5.4	**na**
		6.5	6.3	6.3	6.5	7	6.9	7.2	6.6	6.7	7.9	7.2	5.7	5.7	5.7	5.7	5.5	5.4	5.6	5.9	5.6	
Measuring temperature (pH-H_2O)	°C	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	**na**
		19	19	19	19	19	20	19	19	19	19	19	20	19	19	19	19	19	19	19	19	
Kjeldahl Nitrogen (N)	mg/kg dm	1.1	0.8	1.3	1.1	1.3	1.1	0.8	0.9	1.4	1.2	1	1.2	1.4	1.2	1.1	1.4	1.1	1.2	1	0.9	**na**
		1.6	1.2	1.2	1.9	1.2	1.2	1.4	0.7	1.2	1.2	1.1	1.2	1.5	0.7	1.2	1.1	1.1	1.2	0.9	0.8	

Table NS8.2 – Descriptive statistics of variables

Parameter	Sample	Detects	Mean	Median	Variance	Standard Deviation	Coefficient of Variance	Range	Interquartile Range	UCL95 (Normal-Student's t)	Skewness	Kurtosis	Minimum Detect	Maximum Detect
As	**1**	20	42.40	30.50	1599.20	39.99	0.94	179	29	57.86	2.87	10.05	11	190
	2	20	33.12	26.50	536.86	23.17	0.70	81.60	19	42.08	1.29	0.85	6.4	88
Ba	**1**	19	60.08	33	5129.38	71.62	1.19	332.50	40	87.77	3.42	13.40	17	340
	2	18	42.03	32.50	785.01	28.02	0.67	112.50	32	52.86	1.19	1.62	17	120
CaO	**1**	20	2391.55	1450	7500000	2739.22	1.15	11950	2230	3450.70	2.48	7.68	50	12000
	2	20	1598.50	900	2607550	1614.79	1.01	6850	1250	2222.88	2.47	7.30	350	7200
Cr	**1**	12	4.67	5.25	3.77	1.94	0.42	5.90	3.40	5.42	0.04	-1.34	5.2	8.4
	2	9	3.89	2.50	2.53	1.59	0.41	3.70	2.80	4.50	0.29	-2.02	5.1	6.2
Cu	**1**	20	14.35	14.50	16.55	4.07	0.28	16.30	3	15.92	0.25	0.56	5.7	22.0
	2	20	14	15	9.03	3.01	0.21	12.40	3	15.16	-1.17	2.01	5.6	18.0
Dry Matter	**1**	20	92.99	92.90	2.80	1.67	0.02	6.50	2.30	93.63	0.44	-0.25	90.3	96.8
	2	20	82.48	83.05	16.48	4.06	0.05	17.60	6.30	84.04	-1.10	1.71	71.5	89.1
Hg	**1**	20	5.38	2.45	44.22	6.65	1.24	22.71	5.57	7.96	1.88	3.01	0.29	23
	2	20	3.38	1.50	14.25	3.77	1.12	13.77	2.98	4.84	1.44	1.76	0.23	14
K_2O	**1**	20	0.47	0.49	0.01	0.11	0.24	0.45	0.16	0.51	-0.16	-0.22	0.24	0.69
	2	20	0.48	0.48	0.02	0.13	0.26	0.43	0.15	0.53	0.25	-0.50	0.27	0.70
MgO	**1**	20	984.50	975	71100	266.61	0.27	1080	370	1087.59	0.60	0.68	520	1600
	2	20	932.00	915	62648.42	250.30	0.27	820	390	1028.78	-0.04	-0.99	480	1300
N	**1**	20	1.13	1.10	0.03	0.18	0.16	0.6	0.3	1.20	-0.19	-0.70	0.8	1.4
	2	20	1.18	1.20	0.08	0.29	0.24	1.20	0.30	1.29	0.48	1.29	0.70	1.9
Na_2O	**1**	18	99.75	100	1698.62	41.21	0.41	135	54	115.69	-0.31	-0.75	52	160
	2	20	174.05	180	1877.52	43.33	0.25	159	60	190.80	-0.56	-0.14	71	230
P	**1**	18	0.15	0.15	0	0.06	0.39	0.23	0.04	0.17	-0.43	0.86	0.09	0.25
	2	20	0.16	0.17	0	0.03	0.18	0.11	0.05	0.17	-0.52	-0.10	0.10	0.21
Pb	**1**	20	77.50	50.50	5509.32	74.22	0.96	320	49	106.20	2.57	8.03	20	340
	2	20	58.20	42	2139.43	46.25	0.79	198	39	76.08	2.08	5.35	12	210
pH	**1**	20	6.19	5.85	0.87	0.93	0.15	2.6	1.5	6.55	0.47	-1.47	5.2	7.8
	2	20	6.30	6.30	0.49	0.70	0.11	2.50	1	6.57	0.59	-0.45	5.4	7.9
S	**1**	14	0.24	0.24	0.01	0.11	0.47	0.31	0.18	0.29	0.03	-1.32	0.22	0.41
	2	8	0.16	0.10	0.01	0.08	0.52	0.23	0.12	0.20	0.77	-1.12	0.20	0.33
Sb	**1**	3	1.32	1	0.82	0.91	0.69	3.80	0	1.67	3.42	12.42	2.1	4.8

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

	2	1	1.09	1	0.16	0.40	0.37	1.80	0	1.25	4.47	20.00	2.8	2.8
Se	**1**	3	1.30	1	0.77	0.87	0.67	3.70	0	1.64	3.50	13.09	2.1	4.7
	2	1	1.10	1	0.18	0.42	0.39	1.90	0	1.26	4.47	20.00	2.9	2.9
SO_4	**1**	14	0.72	0.72	0.11	0.34	0.47	0.90	0.55	0.85	0.00	-1.34	0.66	1.2
	2	8	0.49	0.30	0.07	0.26	0.52	0.70	0.37	0.59	0.78	-1.09	0.60	1
TOC	**1**	20	15.20	14	9.54	3.09	0.20	10	5	16.39	0.49	-0.74	11	21
	2	20	15.78	15	22.46	4.74	0.30	17.40	6	17.61	0.48	-0.55	8.6	26
V	**1**	18	7.09	7.25	4.34	2.08	0.29	8.50	2.40	7.89	-0.67	1.14	5.5	11
	2	19	7.41	7.30	3.46	1.86	0.25	8.50	1.60	8.13	-0.56	1.63	5	11
Zn	**1**	20	88.55	84	1035.10	32.17	0.36	128	36	100.99	0.55	0.27	32	160
	2	20	83.60	83.50	481.83	21.95	0.26	82	29	92.09	-0.18	-0.47	38	120

Table NS8.3 – Pearson correlation coefficient matrix between variables - * $p < 0.05$) – first sample series

	Dm	TOC	As	Ba	CaO	Cr	Cu	Hg	Pb	Sb	Se	V	Zn	P	S	SO4	K2O	MgO	Na2O	pH	N
Dm	1.00	-0.21	0.34	0.41	0.36	-0.19	-0.01	0.50	0.36	0.20	0.18	-0.16	0.15	-0.10	-0.06	-0.08	-0.07	-0.09	0.01	0.52*	-0.21
TOC	-0.21	1.00	0.10	0.02	0.11	-0.03	0.19	0.13	0.07	-0.26	-0.25	0.32	0.06	0.37	0.34	0.36	0.30	0.13	0.10	-0.06	0.56*
As	0.34	0.10	1.00	0.98*	0.95*	-0.03	0.47	0.93*	0.99*	0.67*	0.65	-0.04	0.39	0.31	0.35	0.34	0.04	-0.12	0.19	0.85*	-0.22
Ba	0.41	0.02	0.98*	1.00	0.92*	-0.04	0.48	0.92*	0.98*	0.71*	0.69*	-0.07	0.42	0.25	0.31	0.29	0.04	-0.15	0.20	0.86*	-0.31
CaO	0.36	0.11	0.95*	0.92	1.00	0.02	0.37	0.90*	0.94*	0.61*	0.59	-0.05	0.32	0.23	0.33	0.32	-0.02	-0.10	0.17	0.86*	-0.14
Cr	-0.19	-0.03	-0.03	-0.04	0.02	1.00	0.47	-0.17	0.03	-0.20	-0.21	0.79*	0.54*	0.60*	0.29	0.30	0.65*	0.78*	0.52	-0.27	0.34
Cu	-0.01	0.19	0.47	0.48	0.37	0.47	1.00	0.28	0.50*	0.40	0.37	0.52*	0.95*	0.72*	0.57*	0.56*	0.48	0.35	0.21	0.09	0.11
Hg	0.50*	0.13	0.93*	0.92*	0.90*	-0.17	0.28	1.00	0.92*	0.49	0.47	-0.14	0.20	0.17	0.17	0.15	-0.05	-0.24	0.08	0.91*	-0.15
Pb	0.36	0.07	0.99*	0.98*	0.94*	0.03	0.50	0.92*	1.00	0.65*	0.62*	-0.01	0.44	0.32	0.32	0.30	0.05	-0.12	0.20	0.86*	-0.22
Sb	0.20	-0.26	0.67*	0.71*	0.61*	-0.20	0.40	0.49	0.65*	1.00	1.00	-0.27	0.39	0.09	0.42	0.40	-0.22	-0.29	-0.04	0.48	-0.47
Se	0.18	-0.25	0.65*	0.69*	0.59*	-0.21	0.37	0.47	0.62*	1.00	1.00	-0.28	0.36	0.05	0.40	0.38	-0.22	-0.29	-0.04	0.46	-0.46
V	-0.16	0.32	-0.04	-0.07	-0.05	0.79*	0.52*	-0.14	-0.01	-0.27	-0.28	1.00	0.53*	0.83*	0.56*	0.57*	0.88*	0.90*	0.52*	-0.39	0.37
Zn	0.15	0.06	0.39	0.42	0.32	0.54*	0.95*	0.20	0.44	0.39	0.36	0.53*	1.00	0.67*	0.55*	0.54*	0.46	0.41	0.22	0.07	0.08
P	-0.10	0.37	0.31	0.25	0.23	0.60*	0.72*	0.17	0.32	0.09	0.05	0.83*	0.67*	1.00	0.68*	0.67	0.71*	0.58*	0.49	-0.13	0.37
S	-0.06	0.34	0.35	0.31	0.33	0.29	0.57*	0.17	0.32	0.42	0.40	0.56*	0.55*	0.68*	1.00	1.00	0.46	0.43	0.25	-0.04	-0.01
SO4	-0.08	0.36	0.34	0.29	0.32	0.30	0.56*	0.15	0.30	0.40	0.38	0.57	0.54*	0.67*	1.00	1.00	0.46	0.44	0.25	-0.06	0.00
K2O	-0.07	0.30	0.04	0.04	-0.02	0.65*	0.48	-0.05	0.05	-0.22	-0.22	0.88*	0.46	0.71*	0.46	0.46	1.00	0.86*	0.75	-0.32	0.28
MgO	-0.09	0.13	-0.12	-0.15	-0.10	0.78*	0.35	-0.24	-0.12	-0.29	-0.29	0.90*	0.41	0.58*	0.43	0.44	0.86*	1.00	0.54*	-0.42	0.21
Na2O	0.01	0.10	0.19	0.20	0.17	0.52*	0.21	0.08	0.20	-0.04	-0.04	0.52	0.22	0.49	0.25	0.25	0.75*	0.54*	1.00	-0.06	0.15
pH	0.52*	-0.06	0.85*	0.86*	0.86*	-0.27	0.09	0.91*	0.86*	0.48	0.46	-0.39	0.07	-0.13	-0.04	-0.06	-0.32	-0.42	-0.06	1.00	-0.33
N	-0.21	0.56	-0.22	-0.31	-0.14	0.34	0.11	-0.15	-0.22	-0.47	-0.46	0.37	0.08	0.37	-0.01	0.00	0.28	0.21	0.15	-0.33	1.00

*Table NS8.4 – Pearson correlation coefficient matrix between variables - * (p < 0.05) – second sample series*

	Dm	TOC	As	Ba	CaO	Cr	Cu	Hg	Pb	Sb	Se	V	Zn	P	S	SO4	K2O	MgO	Na2O	pH	N
Dm	1.00	-0.57*	-0.44	-0.42	-0.34	-0.37	-0.29	-0.43	-0.36	0.02	0.02	-0.53*	-0.11	-0.52	-0.65*	-0.65	-0.60	-0.59	-0.28	-0.28	-0.64
TOC	-0.57*	1.00	0.48	0.50*	0.36	0.04	0.00	0.60	0.46	0.04	0.04	0.28	-0.30	0.33	0.45	0.46	0.38	0.28	0.10	0.39	0.53*
As	-0.44	0.48	1.00	0.92*	0.91*	0.03	0.53	0.92*	0.97*	0.42	0.42	0.18	0.36	0.38	0.46	0.46	0.16	0.16	-0.04	0.77*	0.60*
Ba	-0.42	0.50*	0.92*	1.00	0.90*	0.02	0.33	0.93*	0.94*	0.48	0.48*	0.02	0.20	0.23	0.51*	0.51	0.07	0.06	-0.04	0.79*	0.40
CaO	-0.34	0.36	0.91*	0.90*	1.00	-0.16	0.40	0.93*	0.95*	0.54*	0.54*	-0.13	0.31	0.13	0.41	0.41	-0.10	-0.10	-0.10	0.93*	0.42
Cr	-0.37	0.04	0.03	0.02	-0.16	1.00	0.40	-0.10	-0.07	-0.16	-0.16	0.67*	0.45	0.57*	0.25	0.25	0.46	0.56	0.19	-0.40	0.34
Cu	-0.29	0.00	0.53*	0.33	0.40	0.40	1.00	0.31	0.46	0.25	0.25	0.50	0.89*	0.66*	0.17	0.17	0.43	0.53	0.18	0.16	0.58*
Hg	-0.43	0.60	0.92*	0.93*	0.93*	-0.10	0.31	1.00	0.94*	0.39	0.39	0.00	0.13	0.20	0.53*	0.53*	0.03	-0.03	-0.13	0.86*	0.52*
Pb	-0.36	0.46	0.97*	0.94*	0.95*	-0.07	0.46	0.94*	1.00	0.52*	0.52*	0.01	0.33	0.27	0.39	0.39	0.01	0.01	-0.12	0.86*	0.51*
Sb	0.02	0.04	0.42	0.48	0.54*	-0.16	0.25	0.39	0.52*	1.00	1.00	-0.27	0.25	0.02	-0.14	-0.14	-0.30	-0.19	0.01	0.50*	0.04
Se	0.02	0.04	0.42	0.48	0.54*	-0.16	0.25	0.39	0.52*	1.00	1.00	-0.27	0.25	0.02	-0.14	-0.14	-0.30	-0.19	0.01	0.50*	0.04
V	-0.53*	0.28	0.18	0.02	-0.13	0.67*	0.50	0.00	0.01	-0.27	-0.27	1.00	0.37	0.80*	0.36	0.36	0.76*	0.76*	0.37	-0.33	0.62*
Zn	-0.11	-0.30	0.36	0.20	0.31	0.45	0.89*	0.13	0.33	0.25	0.25	0.37	1.00	0.46	0.04	0.04	0.24	0.38	0.07	0.08	0.36
P	-0.52*	0.33	0.38	0.23	0.13	0.57*	0.66*	0.20	0.27	0.02	0.02	0.80*	0.46	1.00	0.44	0.43	0.68*	0.66*	0.44	-0.12	0.83*
S	-0.65*	0.45	0.46	0.51*	0.41	0.25	0.17	0.53*	0.39	-0.14	-0.14	0.36	0.04	0.44	1.00	1.00	0.33	0.24	0.36	0.31	0.51*
SO4	-0.65*	0.46	0.46	0.51*	0.41	0.25	0.17	0.53*	0.39	-0.14	-0.14	0.36	0.04	0.43	1.00	1.00	0.33	0.25	0.35	0.32	0.51*
K2O	-0.60*	0.38	0.16	0.07	-0.10	0.46	0.43	0.03	0.01	-0.30	-0.30	0.76*	0.24	0.68*	0.33	0.33	1.00	0.96*	0.33	-0.23	0.56*
MgO	-0.59*	0.28	0.16	0.06	-0.10	0.56*	0.53*	-0.03	0.01	-0.19	-0.19	0.76*	0.38	0.66*	0.24	0.25	0.96*	1.00	0.35	-0.24	0.51
Na2O	-0.28	0.10	-0.04	-0.04	-0.10	0.19	0.18	-0.13	-0.12	0.01	0.01	0.37	0.07	0.44	0.36	0.35	0.33	0.35	1.00	-0.17	0.12
pH	-0.28	0.39	0.77*	0.79*	0.93*	-0.40	0.16	0.86*	0.86*	0.50*	0.50*	-0.33	0.08	-0.12	0.31	0.32	-0.23	-0.24	-0.17	1.00	0.24
N	-0.64*	0.53*	0.60*	0.40	0.42	0.34	0.58*	0.52*	0.51*	0.04	0.04	0.62	0.36	0.83*	0.51*	0.51*	0.56*	0.51*	0.12	0.24	1.00

After the interpolation of selected variables using interpolation methods, ordinary kriging has shown to be the most accurate method for mapping of metal(loid)s and soil properties. The accuracy of the kriging was verified by the standard errors of estimated kriged values calculated by the cross-validation results. The accuracy of the kriging method was validated. Figures NS8.2 to 20 show the mapping of selected variables using the kriging method. Metal(loid)s, CaO and pH that showed a significant positive correlation are distributed in higher concentrations in the North part of the site and the southern area has lower concentrations. Southern and north-eastern areas are considerably more acidic.

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

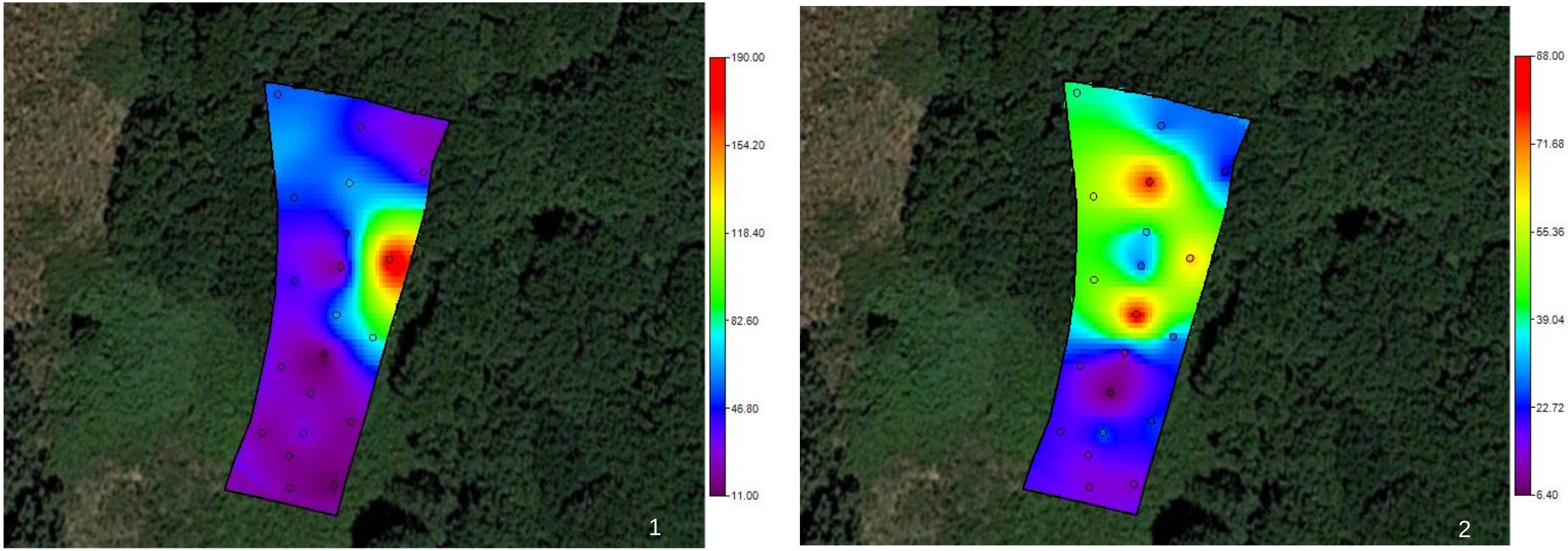


Figure NS8.2 - Spatial distribution and geostatistical interpolation results of Arsenic (mg As/kg) (1 –July 2022; 2 -March 2023)

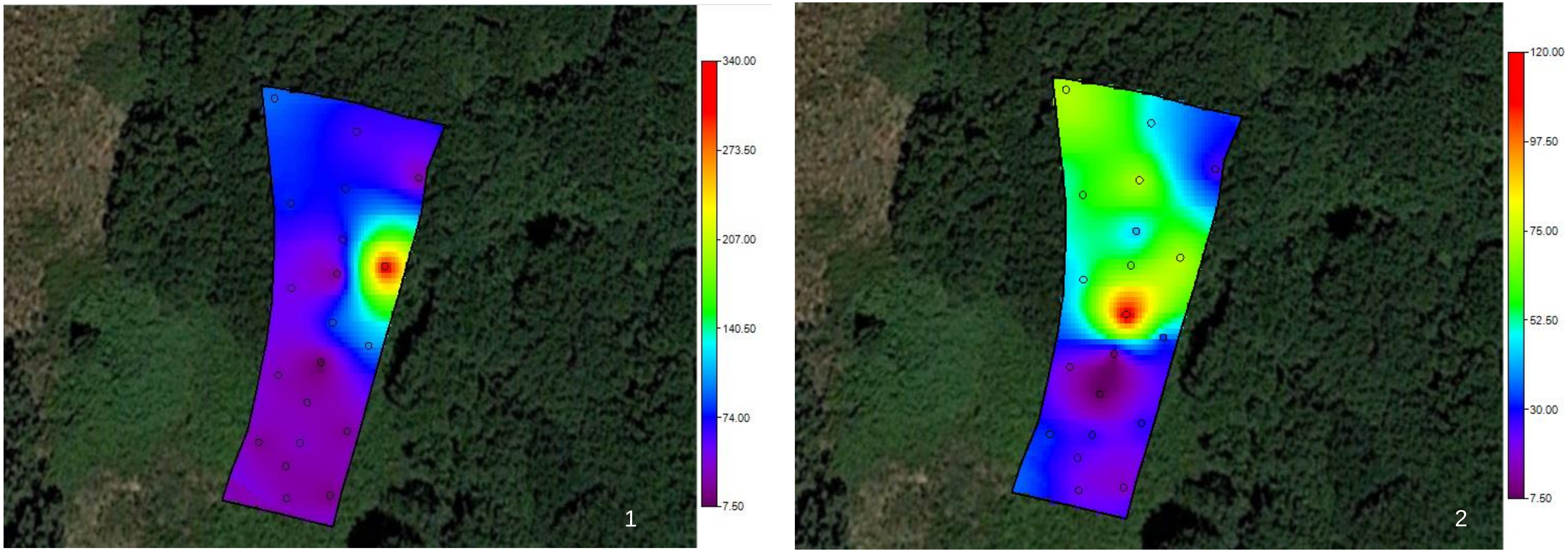


Figure NS8.3 - Spatial distribution and geostatistical interpolation results of Barium (mg Ba/kg) (1 –July 2022; 2 -March 2023)

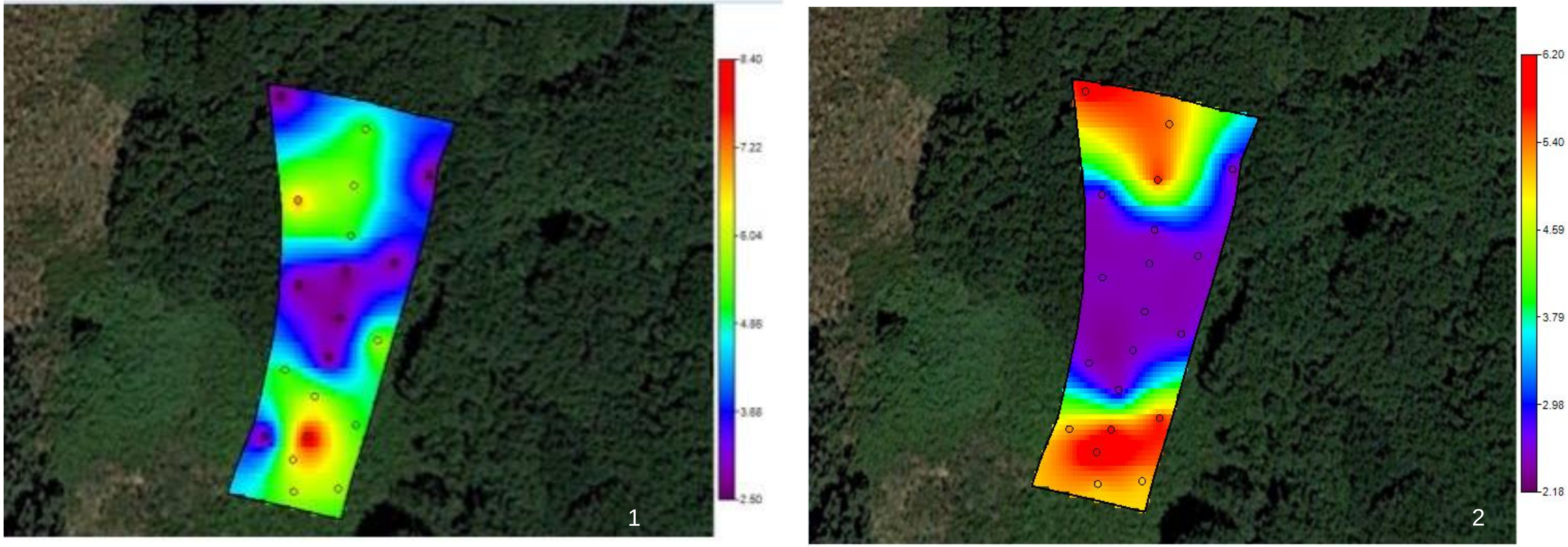


Figure NS8.4 - Spatial distribution and geostatistical interpolation results of Chromium (mg Cr/kg) (1 –July 2022; 2 -March 2023)

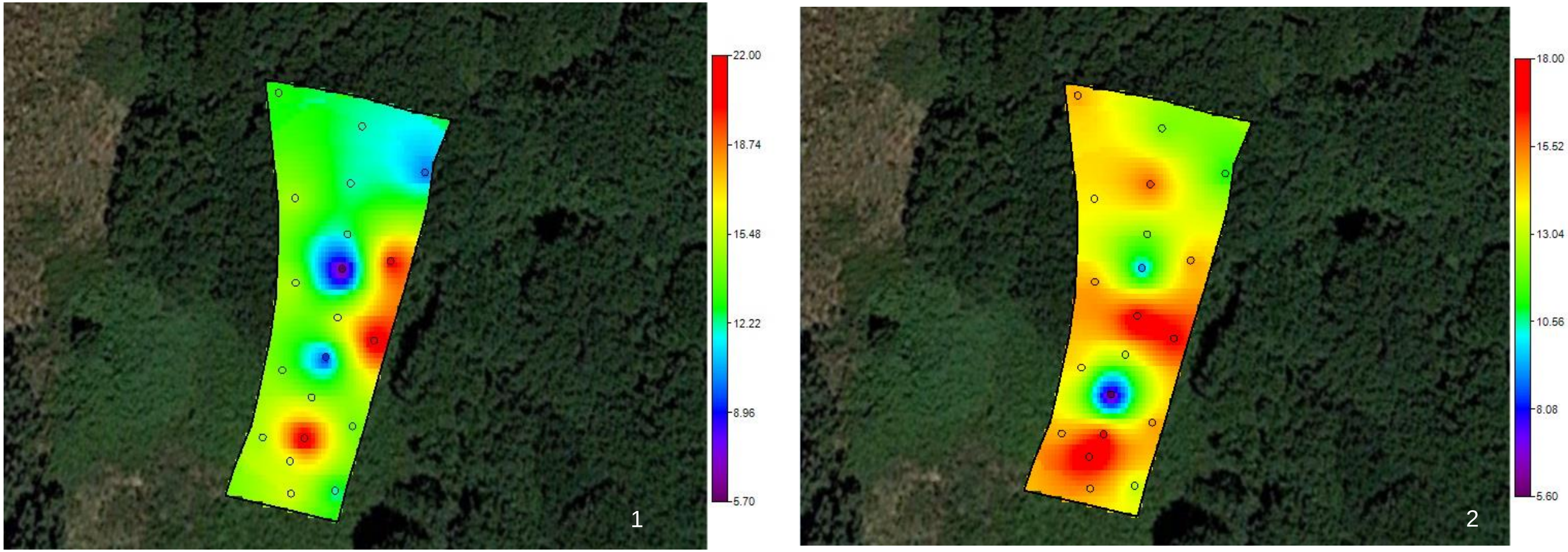


Figure NS8.5 - Spatial distribution and geostatistical interpolation results of Copper (mg Cu/kg (1 –July 2022; 2 -March 2023)

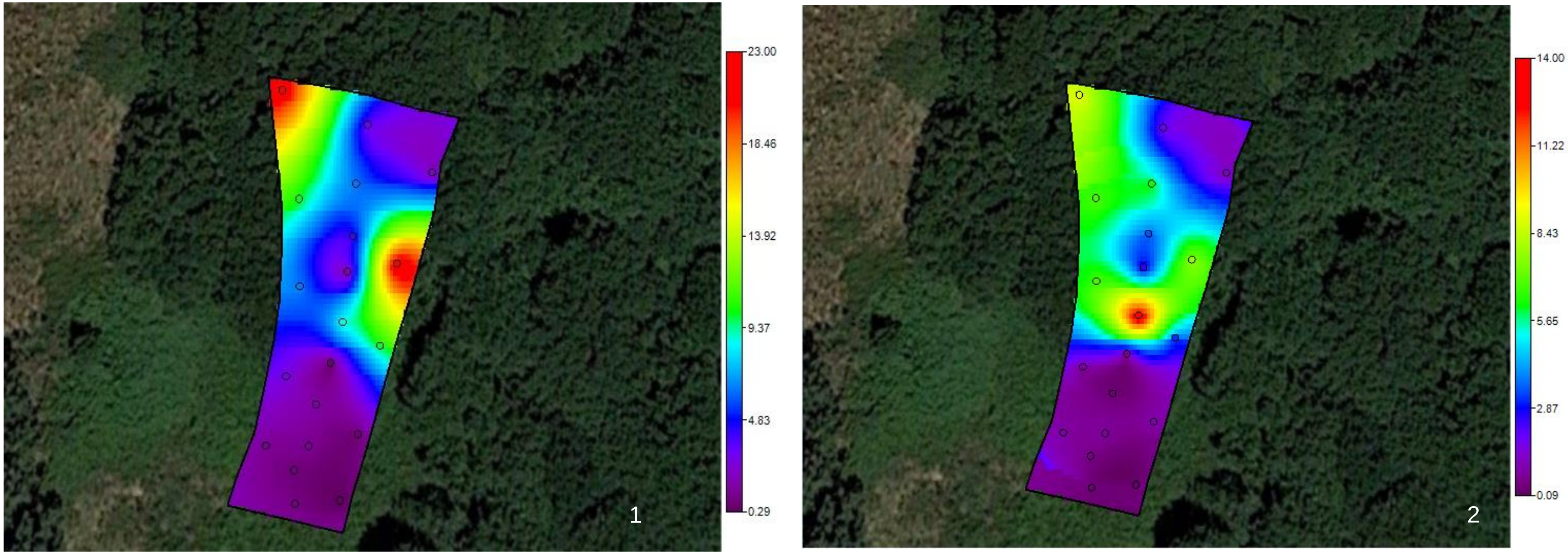


Figure NS8.6 - Spatial distribution and geostatistical interpolation results of Mercury (mg Hg/kg) (1 –July 2022; 2 -March 2023)

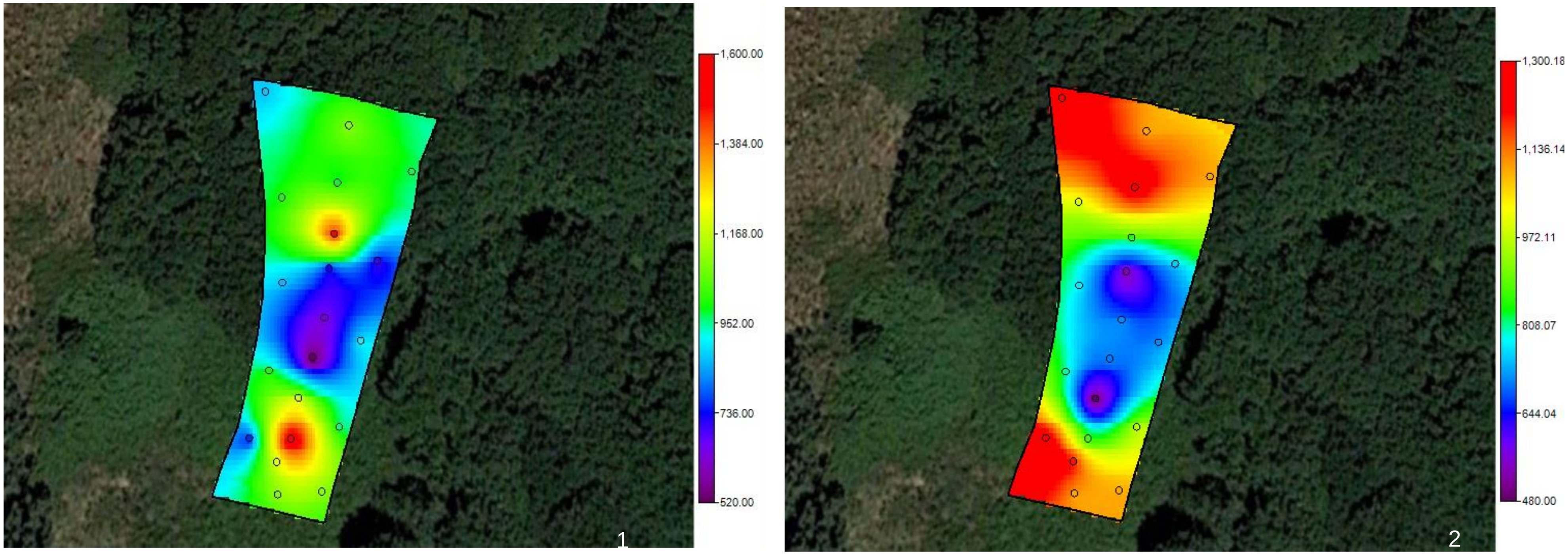


Figure NS8.7 - Spatial distribution and geostatistical interpolation results of MgO (mg/kg) (1 –July 2022; 2 -March 2023)

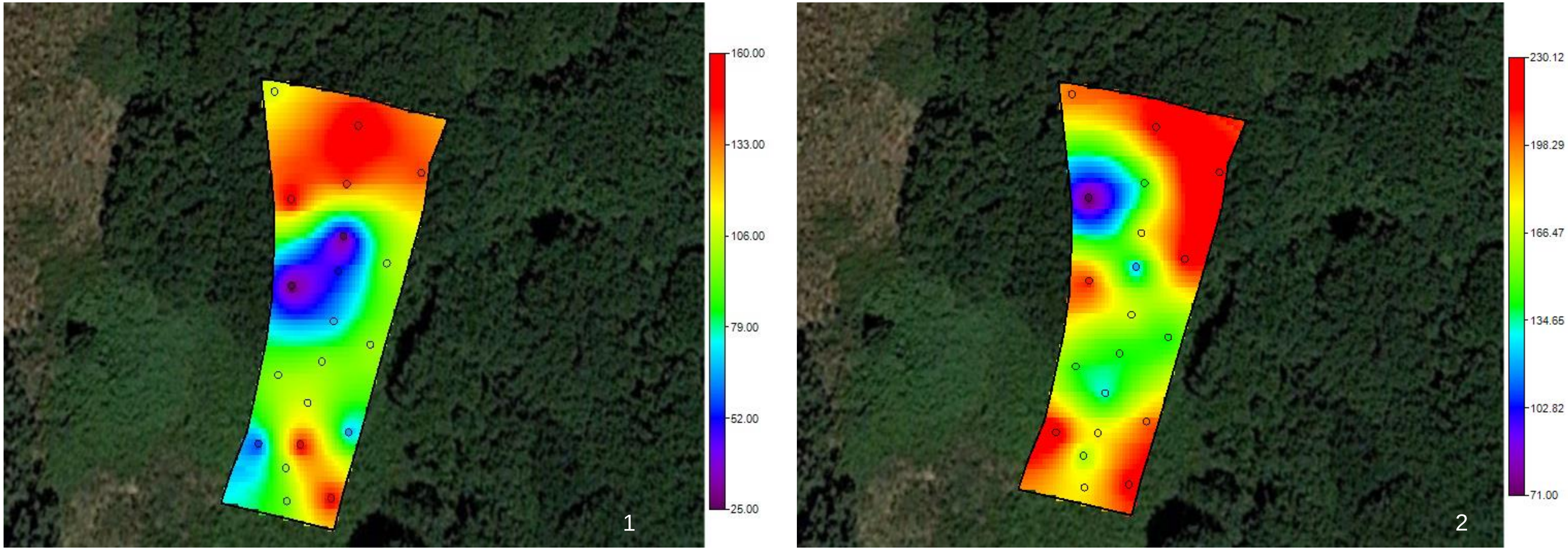
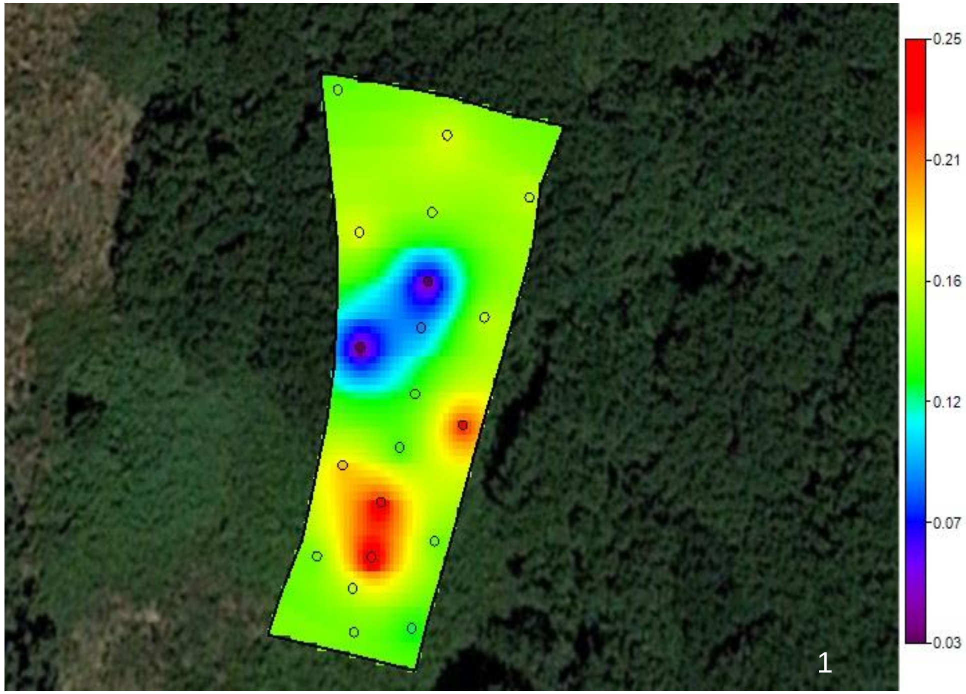


Figure NS8.8 - Spatial distribution and geostatistical interpolation results of Na_2O (mg/kg) (1 –July 2022; 2 -March 2023)



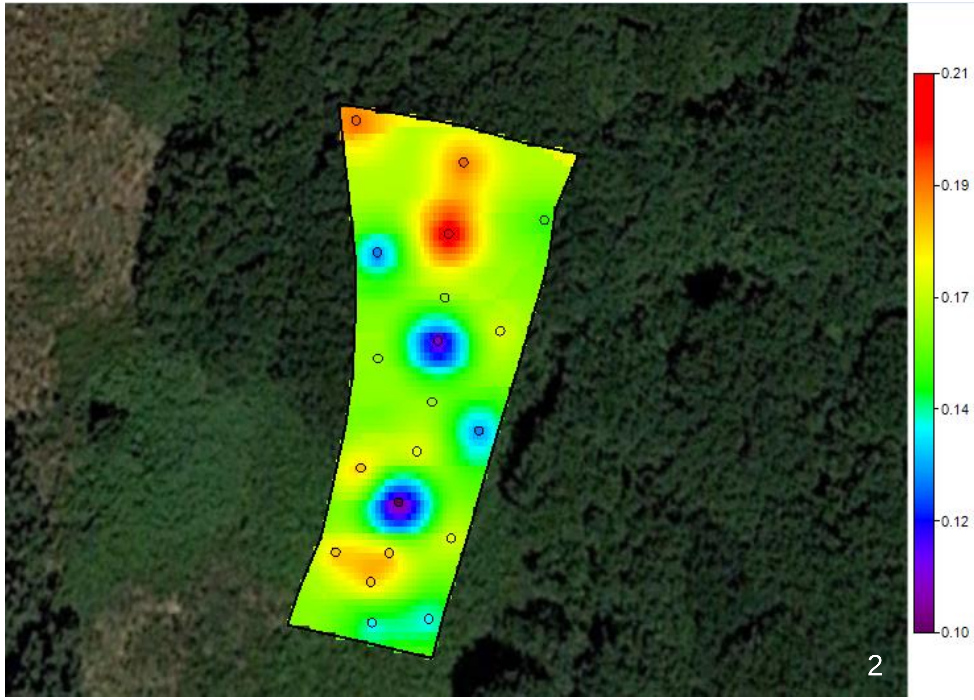


Figure NS8.9 – Spatial distribution and geostatistical interpolation results of P (mg/kg) (1 –July 2022; 2 -March 2023)

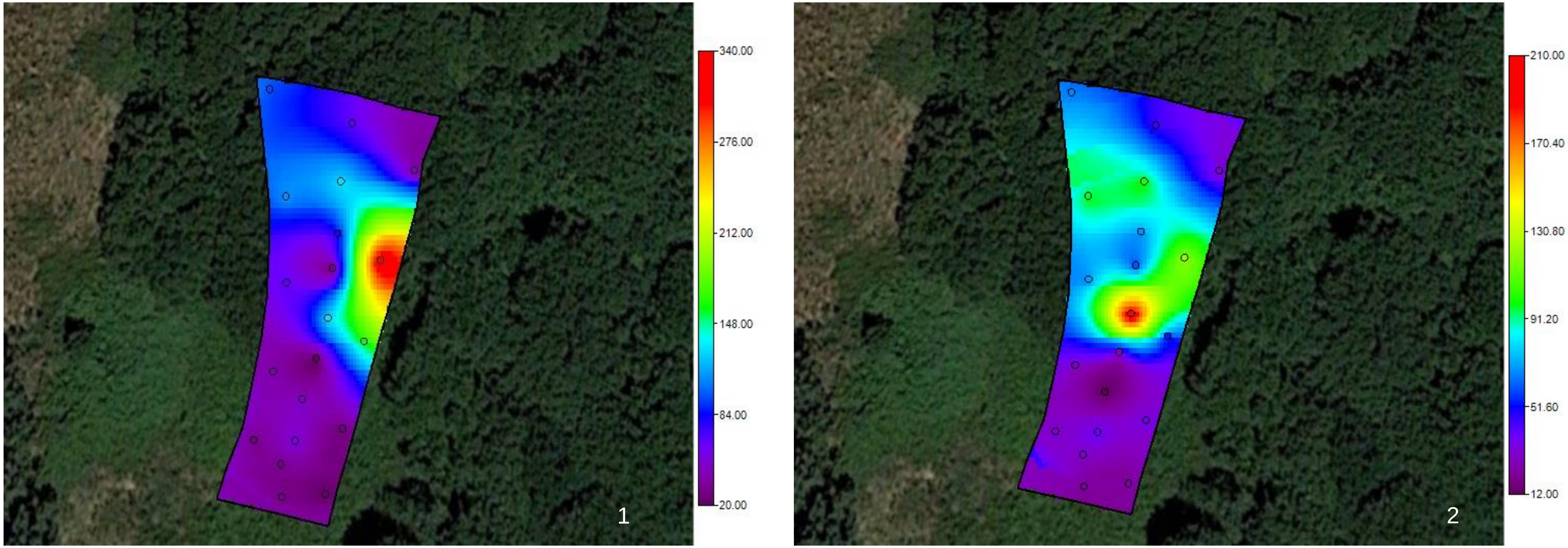
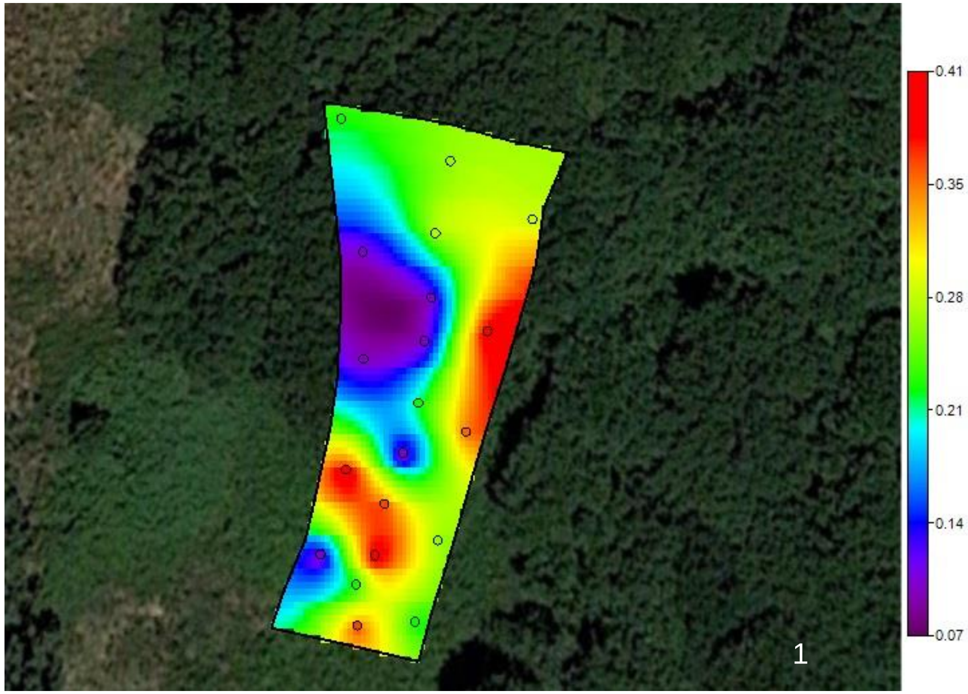


Figure NS8.10 – Spatial distribution and geostatistical interpolation results of Lead (mg Pb/kg) (1 –July 2022; 2 -March 2023)



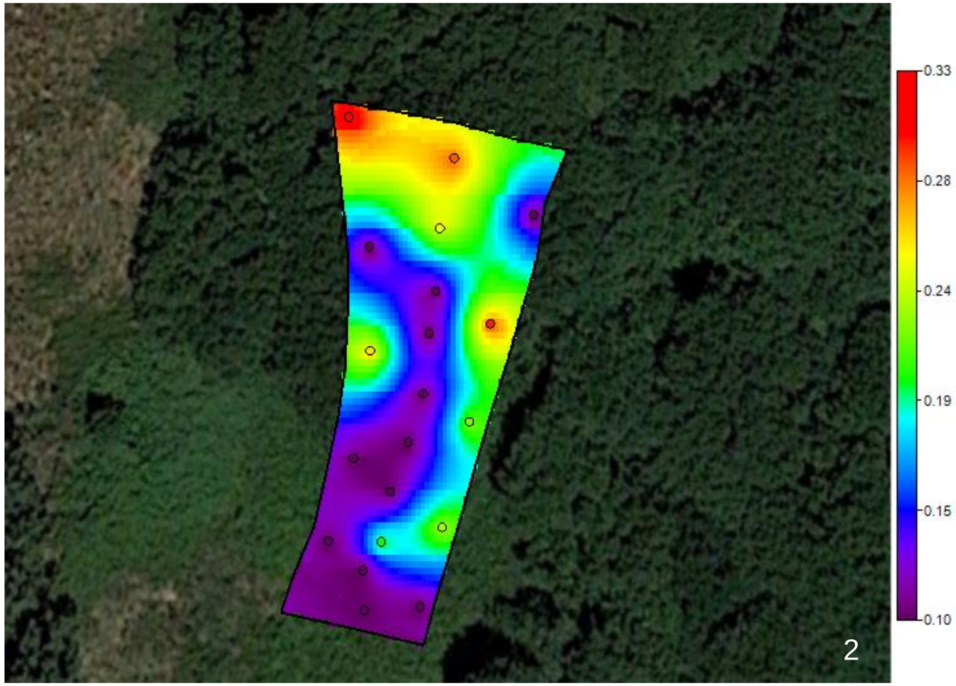


Figure NS8.11 – Spatial distribution and geostatistical interpolation results of Sulphur (mg S/kg) (1 –July 2022; 2 -March 2023)

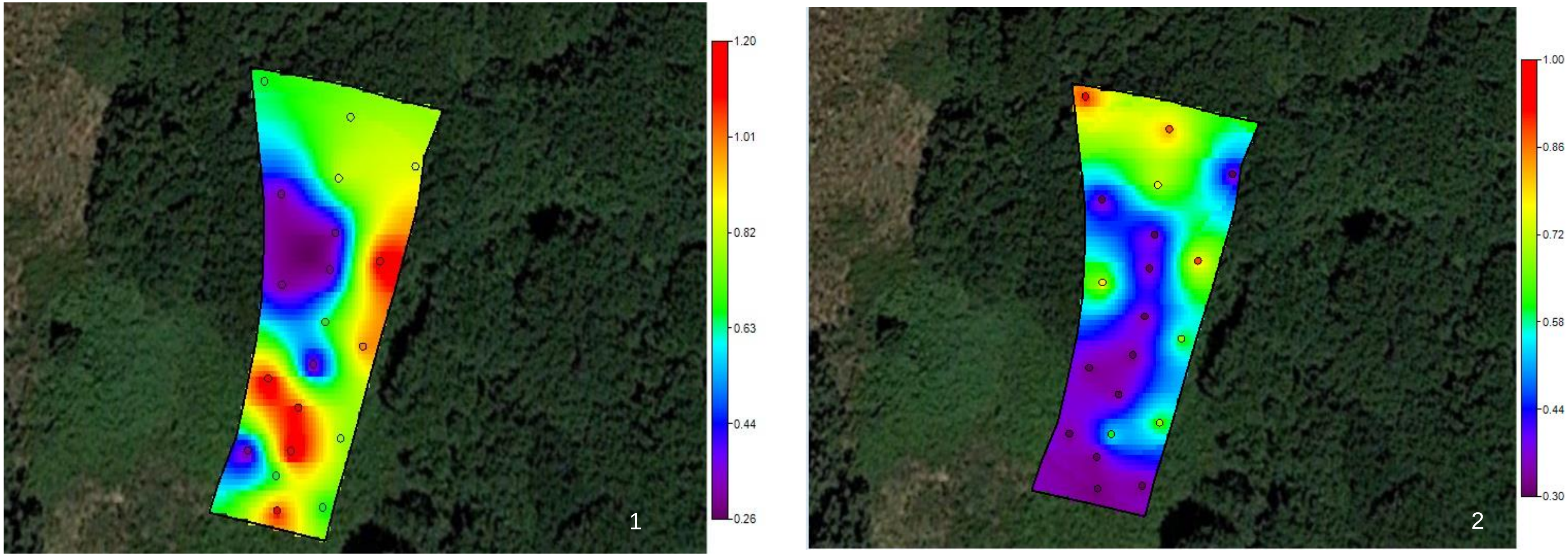
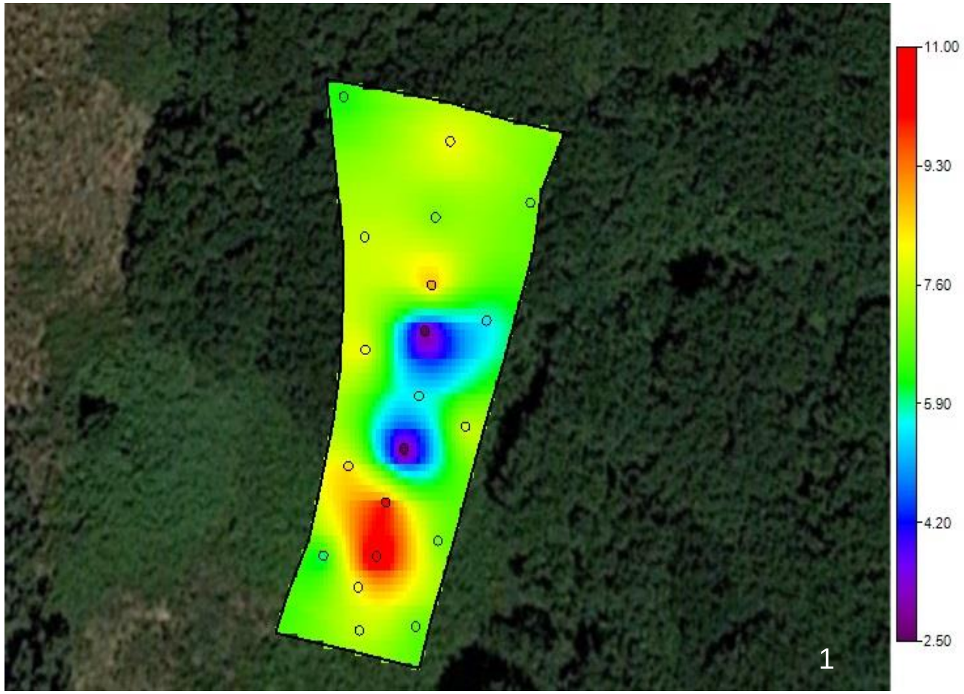


Figure NS8.12 - Spatial distribution and geostatistical interpolation results of Sulphate (mgSO_4/kg) (1 –July 2022; 2 -March 2023)



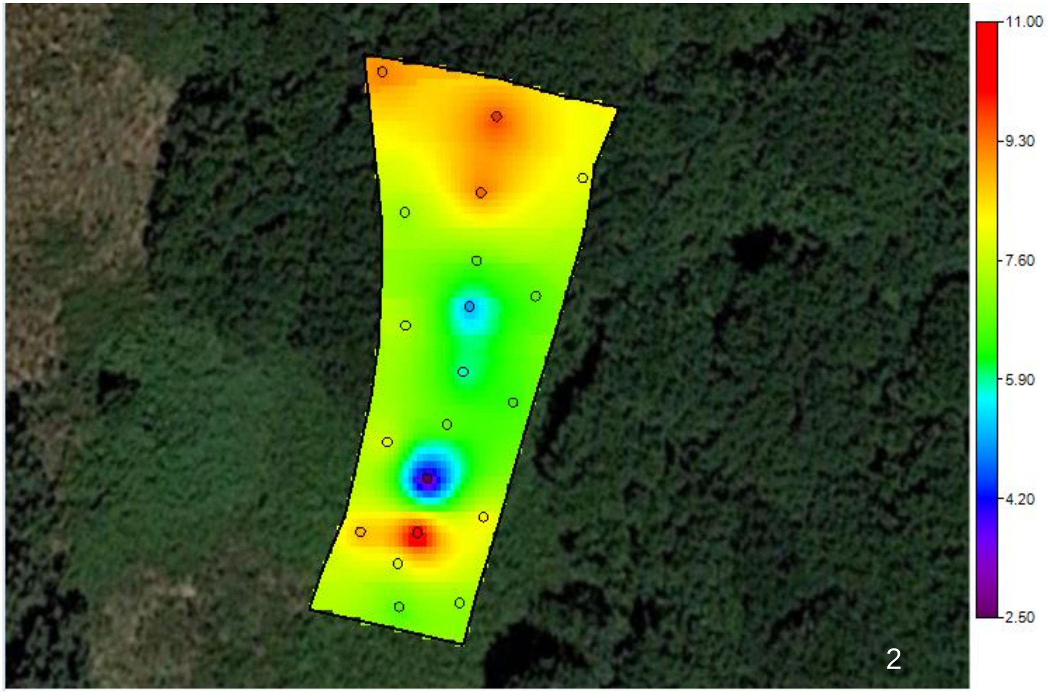


Figure NS8.13 - Spatial distribution and geostatistical interpolation results of Vanadium (mg V/kg) (1 –July 2022; 2 -March 2023)

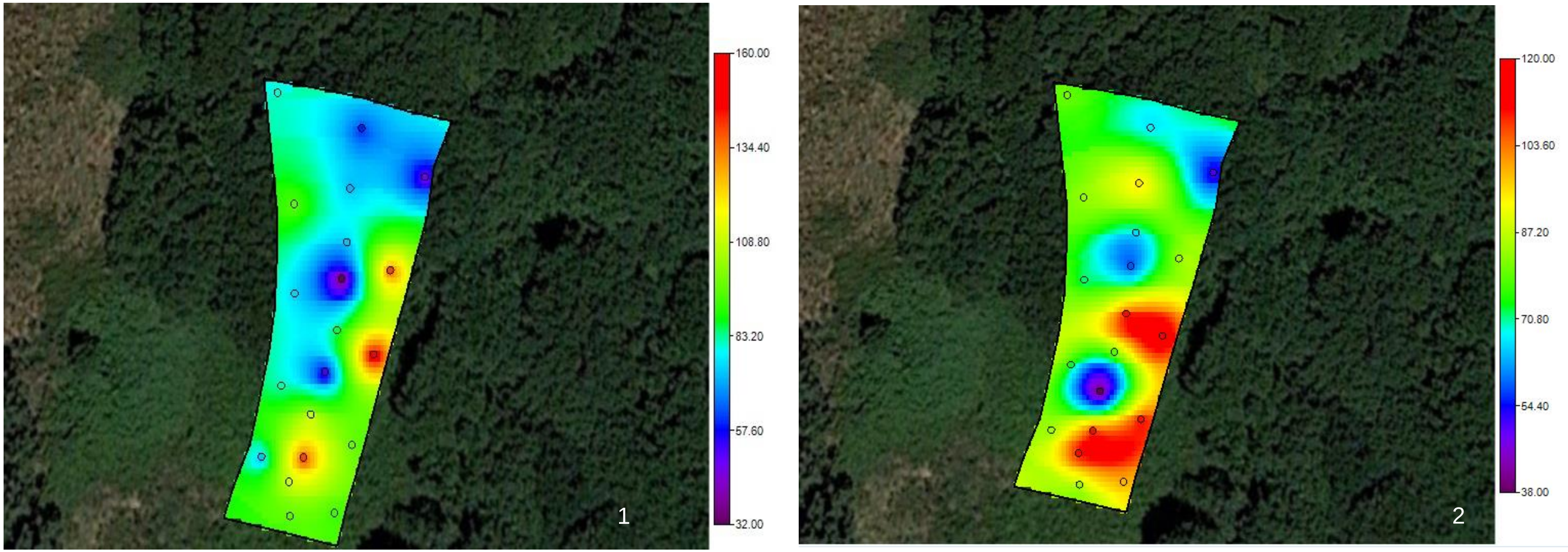


Figure NS8.14 – Spatial distribution and geostatistical interpolation results of Zinc (mg Zn/kg) (1 –July 2022; 2 -March 2023)

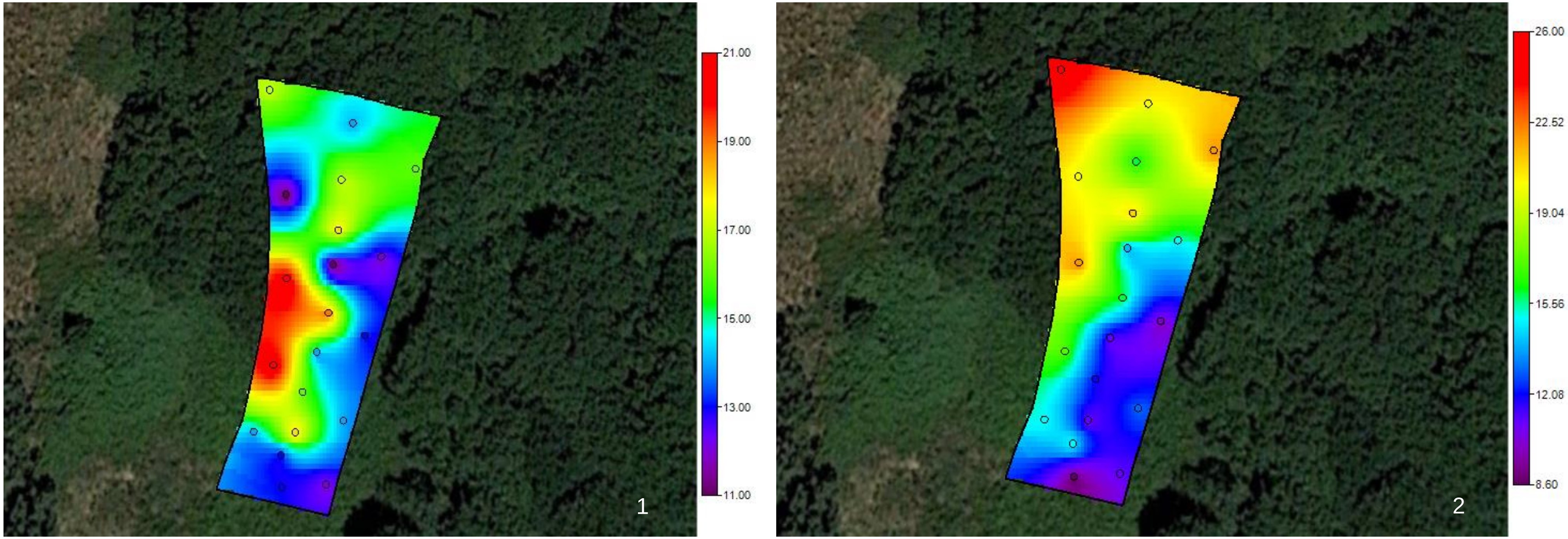


Figure NS8.15 – Spatial distribution and geostatistical interpolation results of TOC (mg C_{org}/kg) (1 –July 2022; 2 -March 2023)

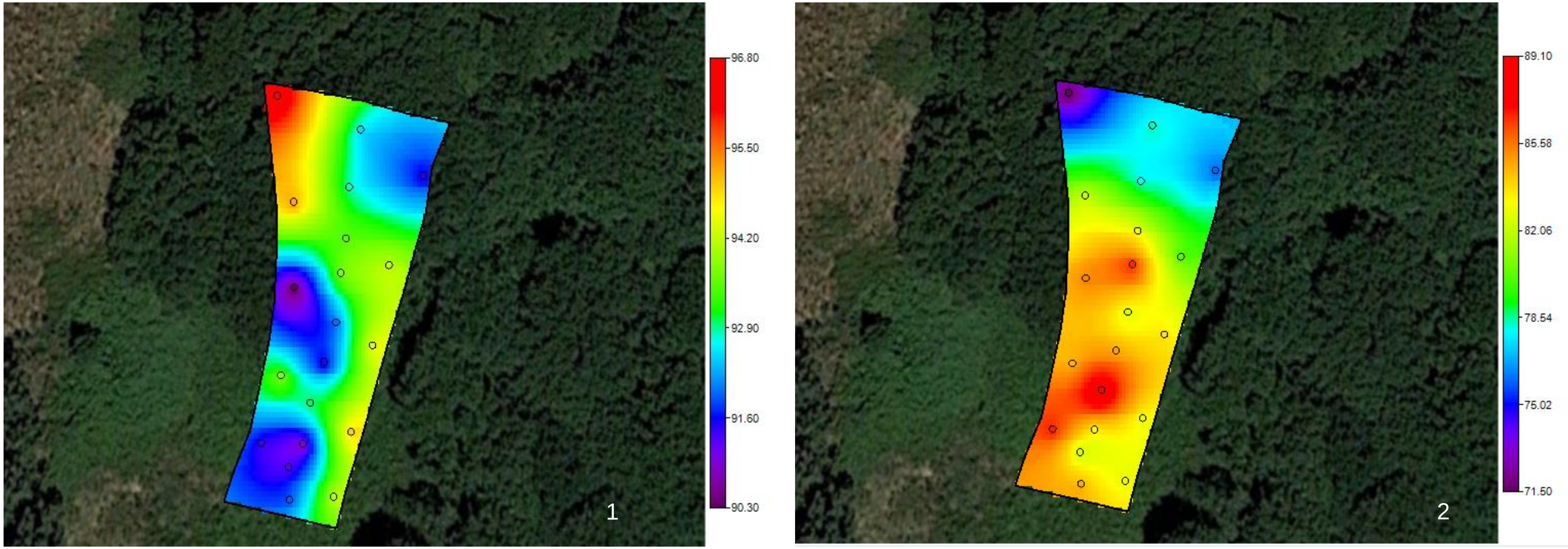


Figure NS8.16 - Spatial distribution and geostatistical interpolation results of Dry Matter (mg/kg) (1 –July 2022; 2 -March 2023)

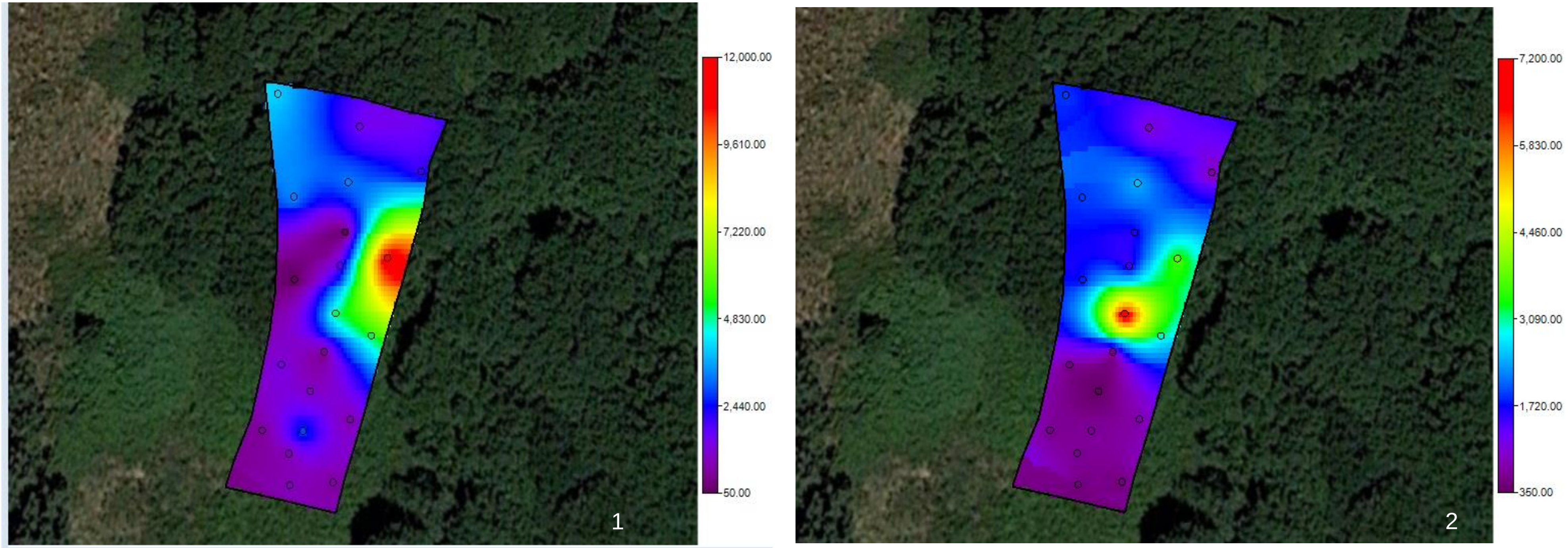


Figure NS8.17- Spatial distribution and geostatistical interpolation results of CaO (mg CaO/kg) (1 –July 2022; 2 -March 2023)

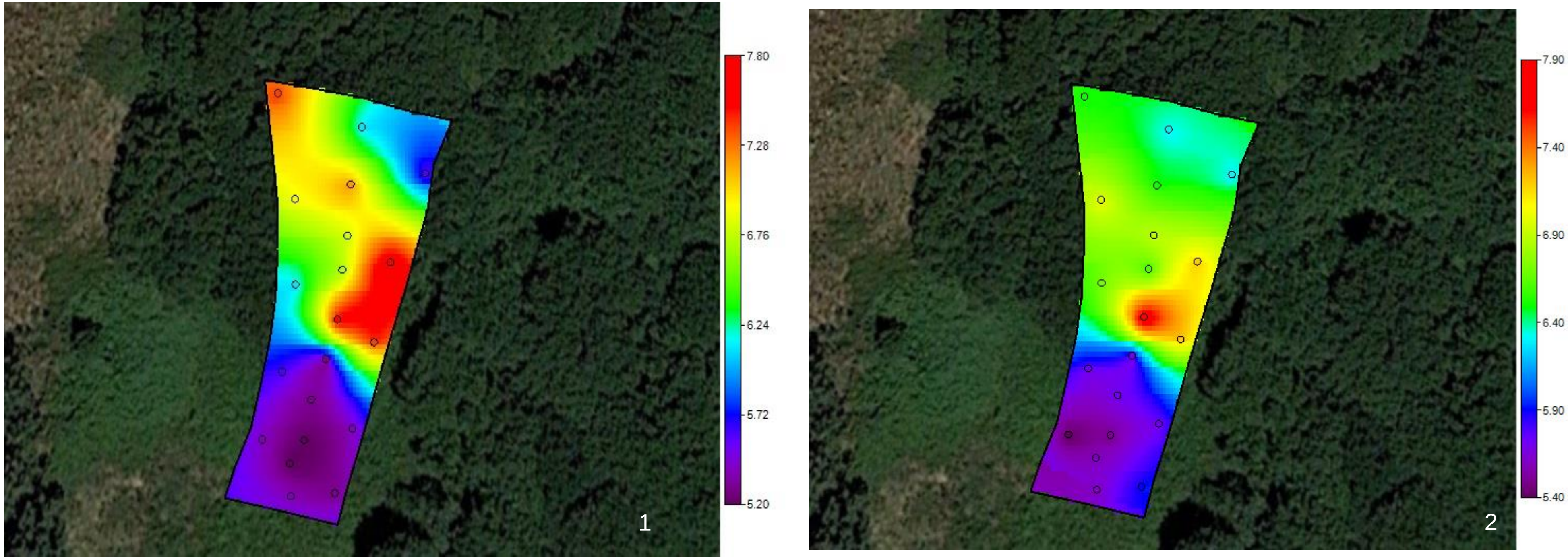
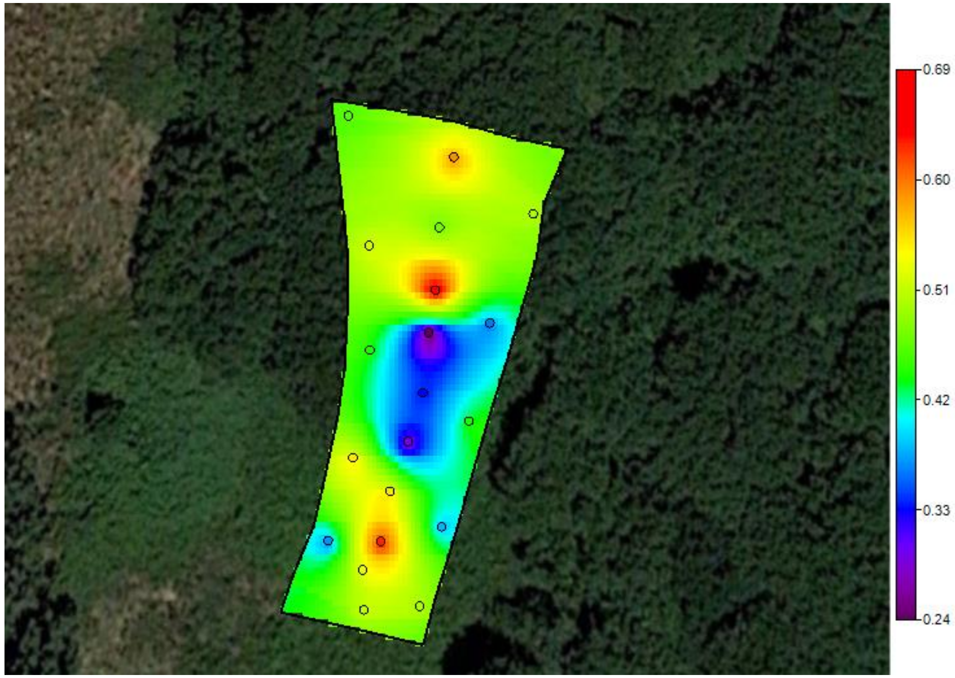


Figure NS8.18 - Spatial distribution and geostatistical interpolation results of pH values (1 –July 2022; 2 -March 2023)



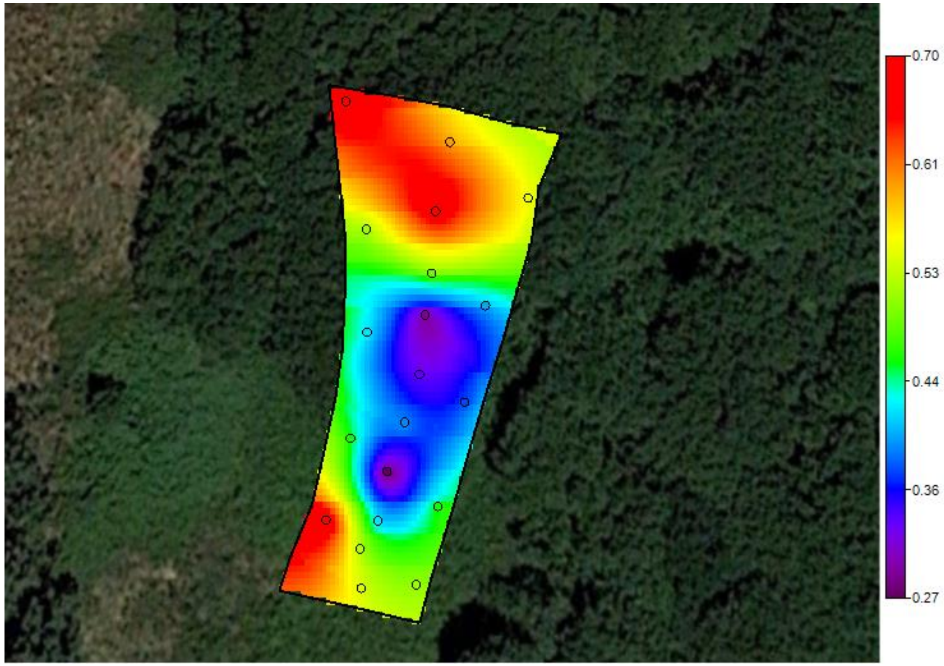


Figure NS8.19- Spatial distribution and geostatistical interpolation results of K_2O (mg k2O/kg) (1 –July 2022; 2 -March 2023)

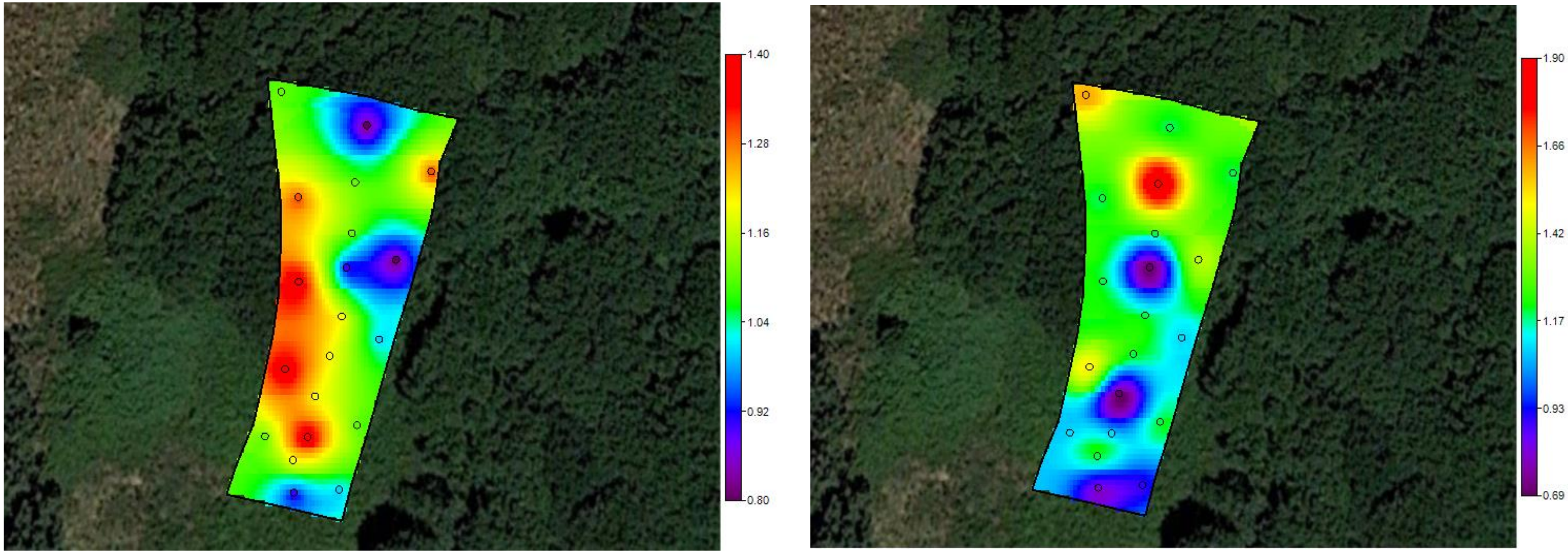


Figure 20- Spatial distribution and geostatistical interpolation results of N (mg N/kg) (1 –July 2022; 2 -March 2023)

Discussion

Although the period of phytoremediation treatment has been short, and experimental data has not yet been fully explored, so effects of specific treatments cannot be fully analysed, nor the influence of the groundwater regime nor other environmental variables can be accounted, it should be noted that the experimental site has experienced notable reductions in the field-average concentration of some elements, indicating a mobilisation of some contaminants, notably, Arsenic (21,9%), Barium (30,4%), Lead (24,7%) and Mercury (36,9%). Such indicators require, naturally, further monitoring over an extended period of phytoremediation.

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

- **Soil macrofauna characterisation**

To characterise the macrofauna of the study area, pitfall traps were used. Pitfall traps consisted of plastic cups with 10 cm high and 8 cm diameter buried at the surface level, with half of its volume filled with antifreeze solution to preserve the collected organisms during the collection period.

Samplings with pitfall traps were carried out in 3 campaigns: the first campaign took place in January of 2022, the second in July of 2022 and the third in March 2023. In the 1st and 2nd campaigns, the pitfall traps were spread in different plots within the study area with the aim to characterise the macrofauna in the Winter (first campaign) and Summer (second campaign) seasons. In the 3rd campaign date, the pitfall traps were placed among the study area and additional pitfall traps were placed in an adjacent and uncontaminated area that was considered as a reference site. The 3rd sampling aimed to compare the macrofauna diversity and richness of the study area (phytomanaged site) with that of an unimpacted area with similar conditions and properties (table NS8.5).
In all sampling periods, a zigzag sampling strategy was followed. Seventeen traps were placed in January (1st campaign), 15 in July (2nd campaign) and 7 in each site in March (3rd campaign).
The traps were collected two weeks after they were placed in the field. The organisms were transferred to plastic containers with a 70% ethanol solution and taken to the laboratory for identification with a Stereo microscope. In the first and second campaigns, organisms were identified to the family level, except organisms of the orders Araneida, Opiliones. Organisms of the Carabidae family were further identified to the species level.
In the third campaign, all organisms were identified to the order level and, within each order, morphotypes were identified to allow comparison between sites. Faunal diversity was assessed by Shannon's diversity index (equation 1), and Pielou's uniformity index (equation 2).

1 - Shannon's diversity index (eq 1)

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where:
H' : biodiversity index
i : species
pi = ni / N ; ni : total number of organisms of a particular species, N : total number of organisms of all species.

Pielou's uniformity index (eq 2)

$$J' = \frac{H}{ln(S)}$$

Where:
H : Shannon-Weiner's diversity
S: total number of species in a sample, across all samples in dataset.

For the first and second sampling dates, the Shannon's diversity index, and Pielou's uniformity index were calculated considering only the identified families in each sampling date.

Table NS8.5- summary of macro-invertebrates sampling strategy

Sample number	Period	Local	Pitfall Traps number	Goal
1	January 2022 (winter)	Study site	17	Soil macrofauna characterization
2	July 2022 (summer)	Study site	15	Soil macrofauna characterization
3	March 2023 (winter)	Study site and uncontaminated adjacent site	7+7	Comparison of macrofauna diversity of the study area with that of an uncontaminated adjacent area

Figure NS8.21 – Sampling and identification of macrofauna using pitfall traps

In samples from January 2022 (first sampling date), a total of 316 organisms belonging to 13 orders were collected, the most abundant orders being Coleoptera (118 individuals), Araneida (85 individuals) and Diptera (77 individuals, accounting for 86% of the sampled organisms. In the samples from July

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

2022 (second sampling date), a total of 312 organisms spread by 11 orders were collected, the most abundant orders being Hymnoptera (92 individuals), Araneida (86 individuals) and Hemiptera (78), accounting for 84% of the sampled organisms. The results of abundance and relative abundance in percentage, considering the classification at the order level, are summarised in figures NS822 and NS.23.
At the family level, some individuals were not identified. No individual of the order Araneida and order Opilione was identified in any of the samples. In the second sampling, there was a significant number of individuals of the orders Hemiptera and Hemynoptera that were not identified (23 and 14, respectively).
In the first sampling date, 23 families were identified. Carabidae was the most abundant family (with 111 organsisms identified) representing 54% of the collected organisms followed by the familiy Dolichopodidae (with 55 organisms). Most families (19 families) were represented by less than 5 individuals. In the second sampling date, 28 families were identified. The Formicidae and Cicadellidae families represented 57% of the collected organisms, with abundances of 59 and 35 individuals, respectively. As in the first sampling date, most of the families (21 families) were represented by less than 5 individuals. The results of abundance and relative abundance in percentage referring to the classification to family level are summarized in figures NS8.24 and NS8.25.
Samples collected in the second sampling date were the most diverse with a Shannon's diversity index of 2.32 and a Pielou's uniformity index of 0.70 while in samples of the first sampling date the Shannon's diversity index was 1.66 and the Pielou's uniformity index was 0.53.

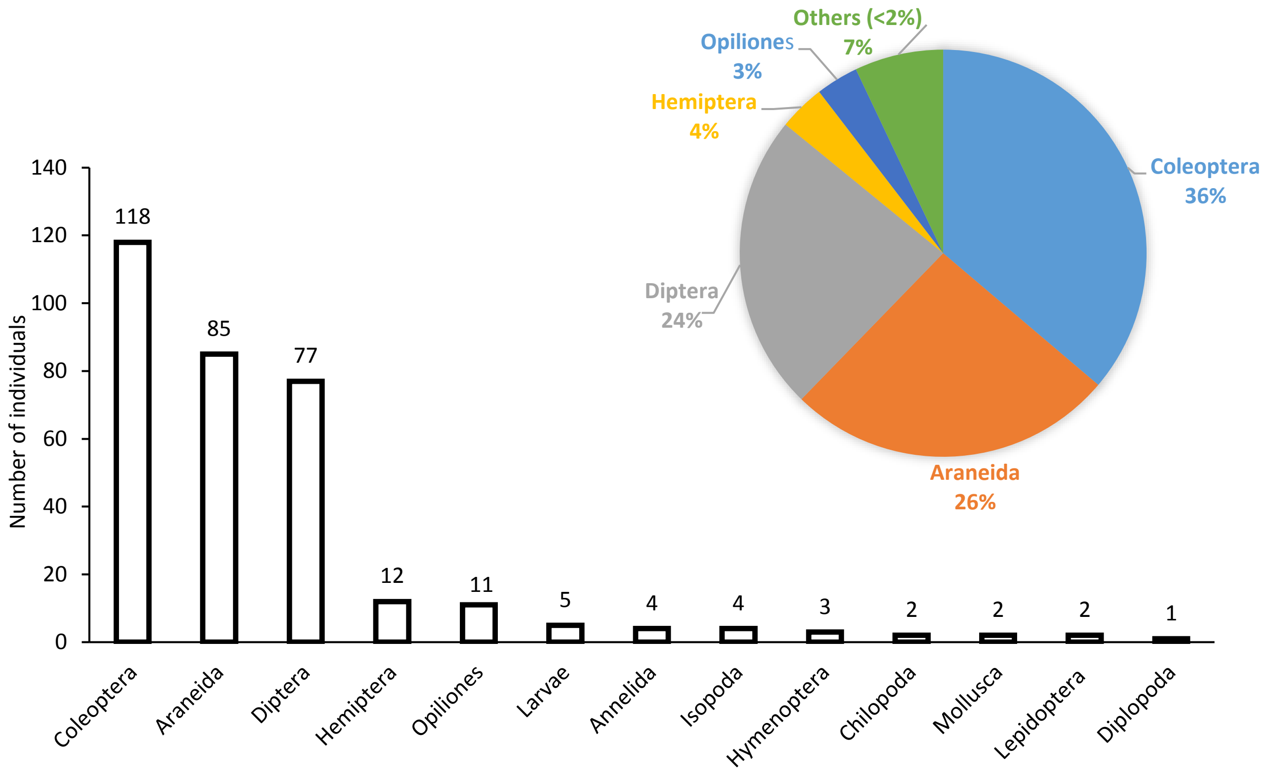


Figure NS8.22 - Abundance (bars chart) and relative abundance (pie chart) of orders collected in pitfall traps inserted in the study site in January 2022 over two weeks (first sampling date).

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

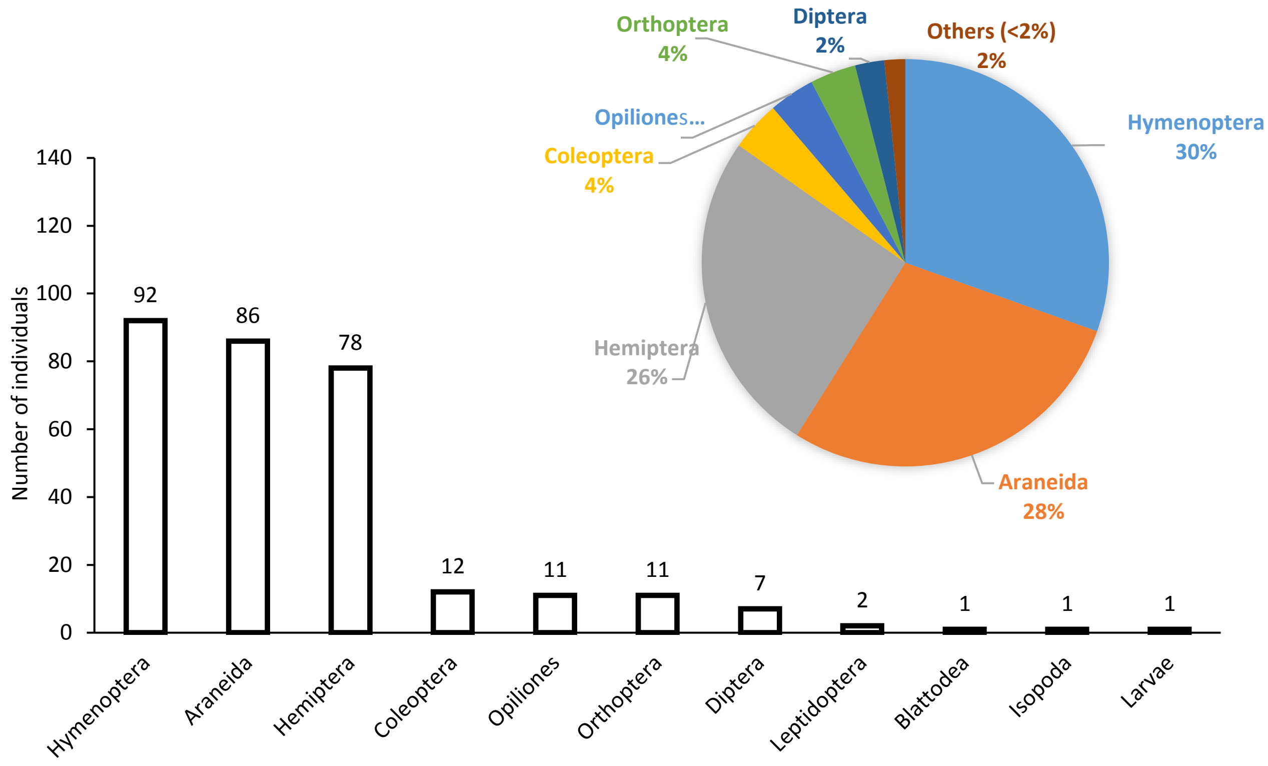


Figure 23 - Abundance (bars chart) and relative abundance (pie chart) of orders collected in pitfall traps inserted in the study site in July 2022 over two weeks (first sampling date).

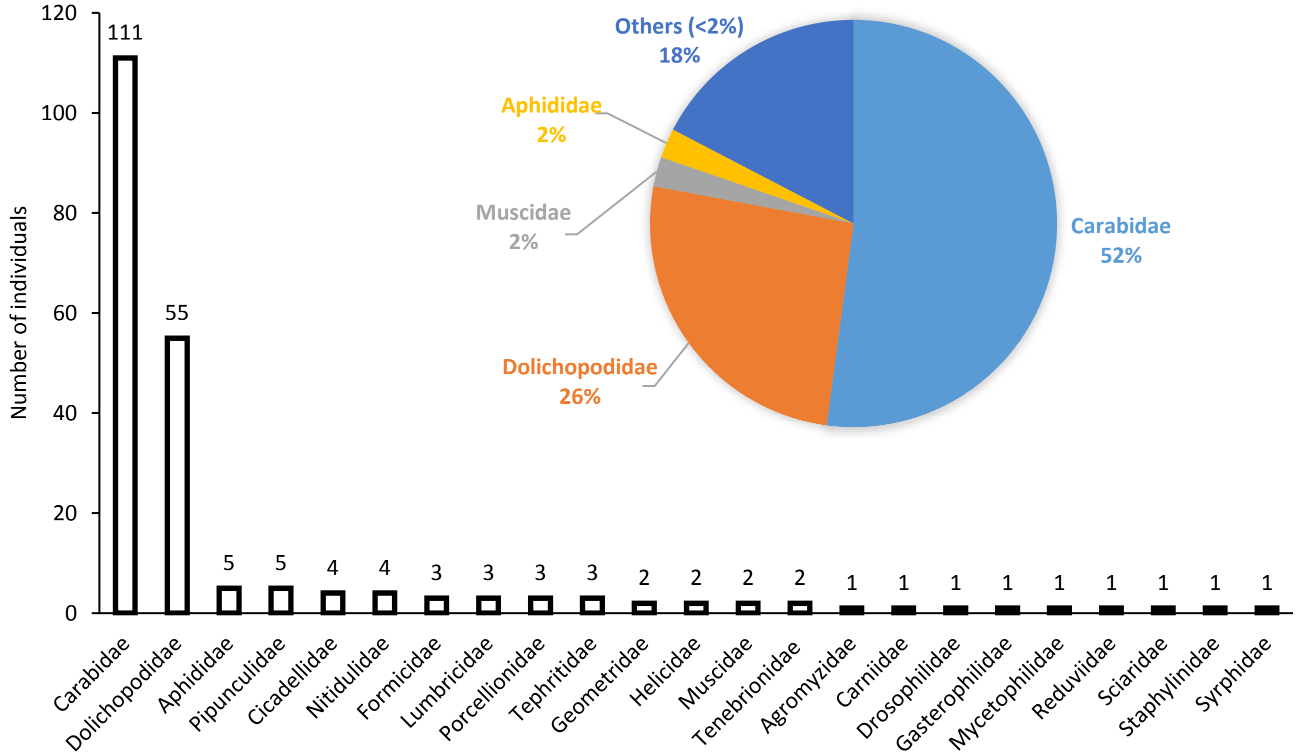


Figure NS8.24 - Abundance (bars chart) and relative abundance (pie chart) of families collected in pitfall traps inserted in the study site in January 2022 over two weeks (first sampling date).

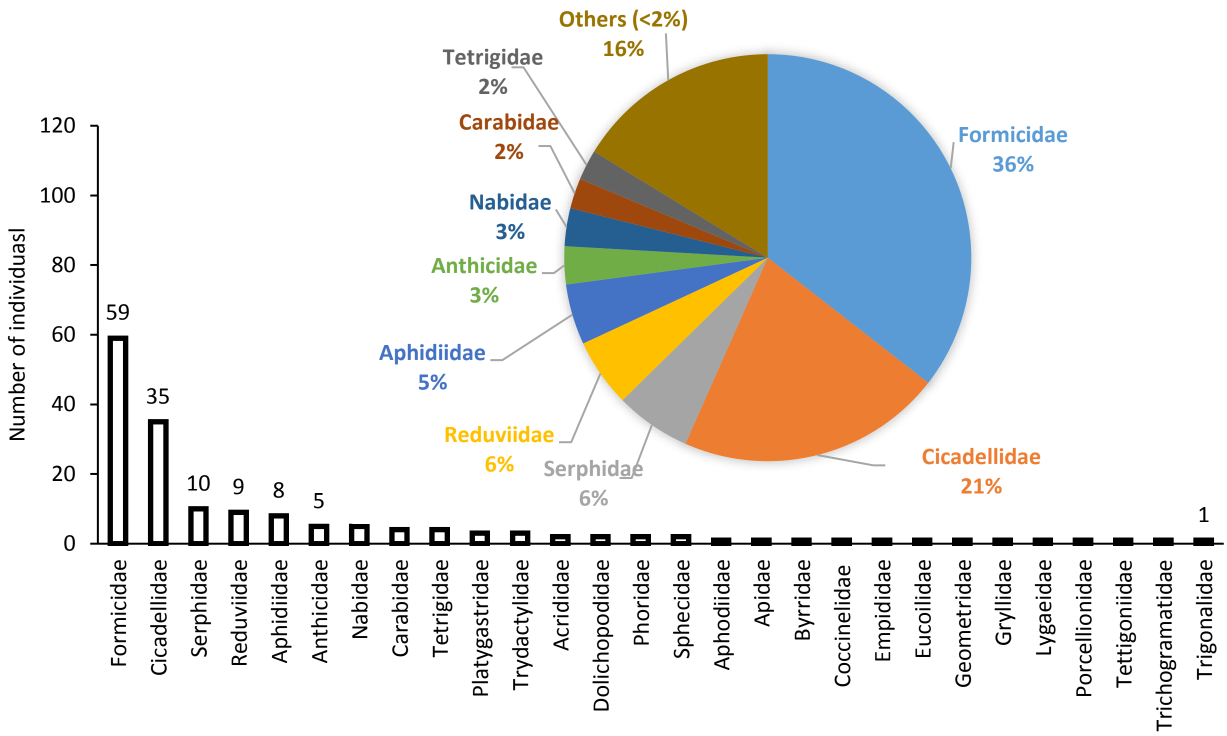


Figure 25 - Abundance (bars chart) and relative abundance (pie chart) of families collected in pitfall traps inserted in the study site in July of 2022 over two weeks (first sampling date).

The species of the specimens belonging to the Carabidae family sampled in the first and second campaigns are presented in table NS8.6. The Carabidae family was the most abundant family in the winter sampling (first campaign) and the most abundant species was *Carabus (Rhabdotocarabus) melancholicus costatus* while in the summer sampling (second campaign) only three species of the family Carabidae were identified.

Table NS8.6- Carabidae species collected in the two sampling periods.

Species	January 2022	July 2022
Asaphidion stierlini (Heyden,1880)	1	0
Carabus (Rhabdotocarabus) melancholicus costatus (Germar, 1825)	72	0
Lophyra (s.str.) flexuosa (Fabricius,1787)	0	2
Nebria((s.str.) salina Fairmaire& Laboulbénè,1854	1	0
Notiophilus marginatus Génè,1859	2	0
Olisthopus elongatus Wollaston,1854	4	0
Pseudophonus rufipes (De Geer, 1774)	0	1
Pterostichus (Pseudomaseus) nigrita (Paykull, 1790)	25	0
Trechus cunicolorum Mequignon, 1931	1	0
Trechus obtusus Erichson, 1837	4	1

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

The abundance of morphotypes identified in samples collected in the third sampling date in the reference and study areas are shown in figures NS8.26 and 27, respectively. In the reference area, a total of 37 individuals were collected while in the study area, 73 individuals were collected. The Areneida order was represented in greater abundance in both sampling sites (54% in the reference area and 78% in the plot). The morphotype 1 of the Araneida order was collected in both sites and was the most abundant morphotype both in the reference and study areas. In a total of 28 identified morphotypes, only 5 were identified in the two areas.

Considering all morphotypes identified, the reference area was the most diverse with a Shannon's diversity index of 2.19 and a Pielou's uniformity index of 0.79 while in the plot site, the Shannon's diversity index was 1.53 and the Pielou's uniformity index was 0.54.

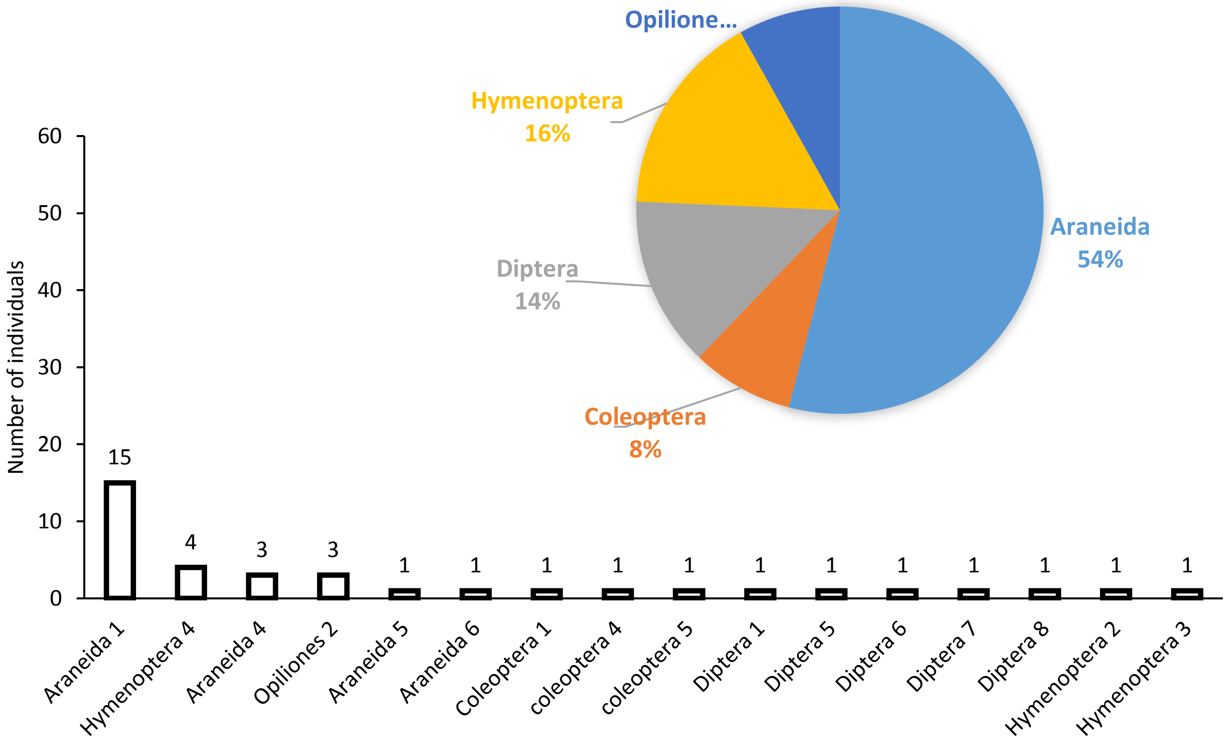


Figure NS8.26 - Abundance (bars chart) and relative abundance (pie chart) of Morphotype collected in pitfall traps inserted in the reference site in March 2023 over two weeks

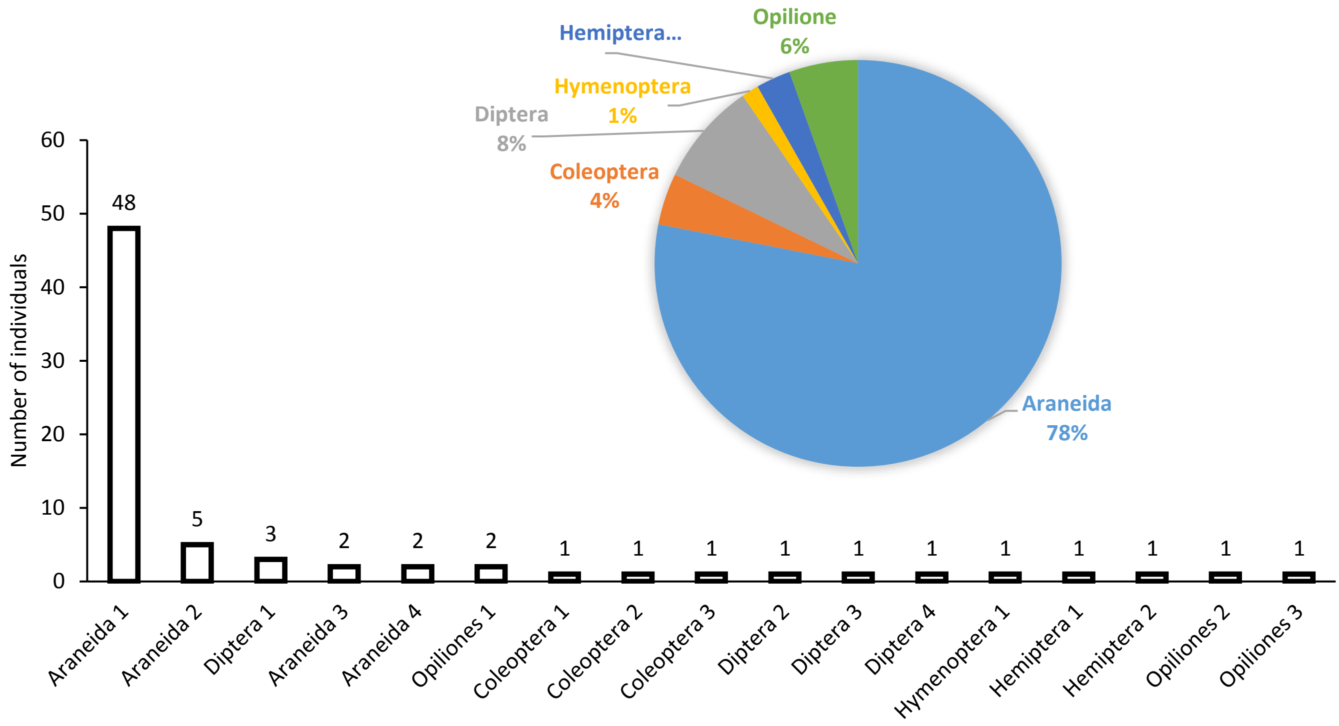


Figure NS8.27 - Abundance (bars chart) and relative abundance (pie chart) of Morphotype collected in pitfall traps inserted in the r study site in March of 2023 over two weeks

Discussion

There was a considerable difference between the communities found in the first and second campaigns in the study area and this difference was evidenced by the fact that, from the total of 43 identified families, only 8 were found on both occasions (Aphidiidae, Carabidae, Cicadellidae, Dolichopodidae, Formicidae, Geometridae, Porcellionidae, and Reduviidae). During winter, the community is dominated by the Carabidae family. According to Oliveira (2016) most species of Carabids in the south Europe are active in the winter months and inactive in the summer months (July, August and September). Many species of Carabids decrease their activity with high temperatures (above 25°C) (Kruse et al., 2008). The most abundant species is *Carabus (Rhabdotocarabus) melancholicus costatus (Germar, 1825)*. This species is observed in Portugal in the north of the Tagus River (J. Serrano, 2013; Aguiar & A. Serrano, 2013). Some works have established a negative influence of pollutants resulting from human activity on the development of several species of Carabids, which makes it possible to use this group as an indicator of metal(loid)s at the study site, albeit, further studies on its use are still required (Butovsky, 1994; 2011). On the other hand, in the summer, the most abundant family was Formicidae with an increased activity at the soil and air temperatures of 20–30°C (Véle et al., 2009)

On the third campaign, the absolute abundances found were generally lower than those observed in the first and second campaigns, but that fact is related to the lower sampling effort adopted in each area of the third sampling date as the number of pitfall traps used per area in the third sampling date was about half of the number of pitfall traps used in the study area in the first and second sampling dates. Regarding the communities of both reference and study areas, both communities were dominated by species of the order Araneidae and just the orders Hemipterae and Opilione were represented only in the study area. Although the highest number of specimens was found in the pitfall traps of the study area, the highest diversity and uniformity was found in communities of the reference area. This happened especially because the communities of the study area were largely dominated by the morphotype 1 of the Araneidae order, while the communities of the reference area had a lower dominance in this morphotype. The lower diversity and uniformity of communities of the study area, when compared to that of the reference area, might be related to soil contamination.

● **Feeding activity of Soil organisms**

Materials and methods

To evaluate the feeding activity of soil dwelling organisms in the study site, the bait lamina method was used based on the procedures described in the ISO 18311 (ISO 2016). Bait laminas consisted of small plastic strips (16 cm length and 0.5 cm wide) with 16 perforations (0.5 cm distant from each other) filled with a bait composed of a mixture of wheat bran, activated charcoal and cellulose in a weight ratio of 27:3:70. This bait is exposed to the soil organisms after inserting the strips vertically into the soil with the aid of a stainless-steel scoop. After a certain period of time into the soil, the strips are carefully removed from the soil and the loss of the bait material is assessed by counting the empty apertures of the bait lamina strips. The number of empty or partially empty holes (i.e. areas from which the bait material has been eaten) as well as their vertical distribution are determined to evaluate the feeding activity of soil organisms and the predominance of that activity in terms of depth.

Bait-lamina were placed in different plots of the study area in two different periods. The first was in summer/ autumn of 2022 and the second in winter/spring of 2023. In summer/autumn, the bait-lamina assay was performed only in the study site to characterize the feeding activity of soil communities in the study area while in winter/spring the bait-lamina assay was performed simultaneously in the study site and in an uncontaminated and adjacent area considered as a reference in terms of soil local communities to allow the comparison between feeding activity of communities from the two areas. In each sampling period, groups of 16 bait lamina were spread in the area to be evaluated. In the summer/autumn period, 14 plots were considered while in the winter/spring campaign, 7 plots in the study area and another 7 plots in the reference area were considered (figure NS8.26). The time period that the bait lamina stayed into the soil depended on the activity of the communities in each sampling period. To evaluate the adequate moment to collect the bait lamina, additional bait lamina were inserted into the soil simultaneously, just to allow to verify the degree of bait eaten every two weeks. The bait lamina were removed from the soil only when the additional bait lamina had at least half of the holes with the bait eaten. Once collected, the bait-lamina sticks were carefully removed from each site to plastic bags, and immediately brought to the laboratory and stored at 4°C until the number of empty or partially empty holes in each stick was recorded. These values were then expressed as the percentages of eaten holes per stic.

Table NS8. 6- Summary of bait lamina sampling strategy

Sample number	Sampling Period	Local	Sampling Points	Exposure time
1	summer/ autumn 2022	Study Site	14	90 days
2	winter/spring 2023	Study Site and uncontaminated adjacent site	7+7	34 days

Figure NS8.28 – Distribution of groups of 16 bait lamina for the sampling periods: summer/autumn (left map) and winter/spring (right map) in the study site or the study and reference sites respectively.

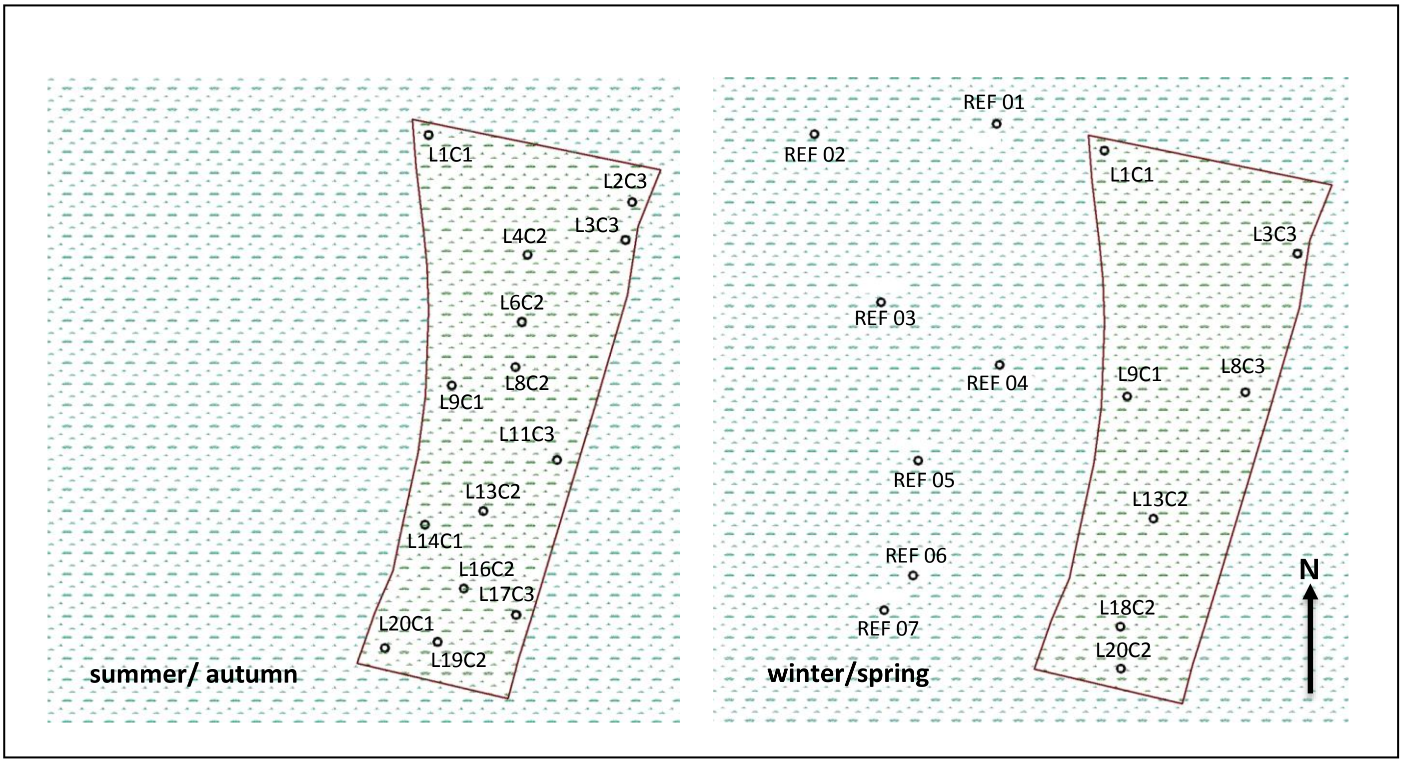


Figure NS8. 29 – Scheme of a bait lamina with empty (white circles), partially empty (partially dark circles) and filled (full dark circles) holes (left draw), a group of 16 bait lamina sticks inserted into the soil (left photo) and a sampled bait lamina (right photo).

Results

The results expressed in average percentage of empty holes, and considering the depth for the periods of summer/autumn and winter/spring, are presented in figure NS8.30 and the results expressed in average percentage of empty holes, and considering the depth for the reference site and the study site, over 34 days are presented in figure NS8.31.

Comparing the feeding activity over the different soil depths between the two sampling periods, in general, the feeding activity observed in summer/autumn showed a tendency to be lower (even in a larger period of exposure of 90 days) than that observed in the winter/spring period (with an exposure period of only 34 days).

Results obtained in the winter/spring in the reference (a total of 7 plots) and study (a total of 6 plots as one bait lamina group was missing) areas were obtained after a period of 34 days. It was evidenced that the reference area has higher feeding activity (59%) at the surface (0-1cm) decreasing in depth to an average of 43% at 7-8 cm depth, while in the study site, there is a higher feeding activity (44%) at the soil surface (0- 1cm) and a lower feeding activity at the 4-5 cm depth (30%).

On the other hand, comparing the feeding activity found in the plots of the study site with that observed in the plots of the reference site (both over an exposure period of 34 days), the feeding activity in the reference plots showed a clear tendency to be higher (on average) than that in the study site in all soil depths evaluated.

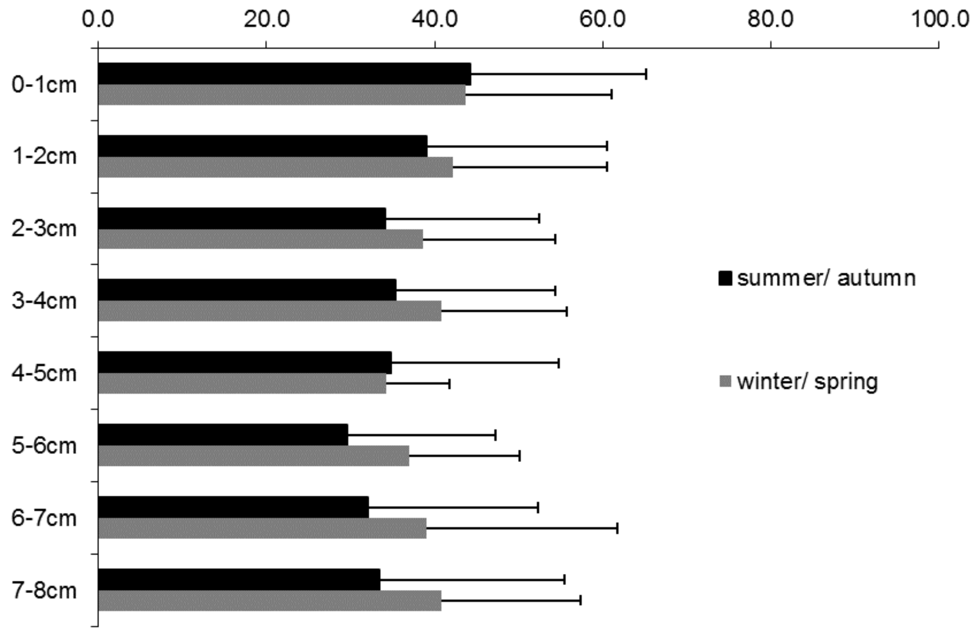


Figure NS8.30 – Average of percentage of totally or partially eaten holes (mean ± standard deviation) of a group of 16 bait lamina in the different depths, for the different sampling plots spread in the study site exposed during the summer/autumn over 90 days and during the winter/spring over 34 days

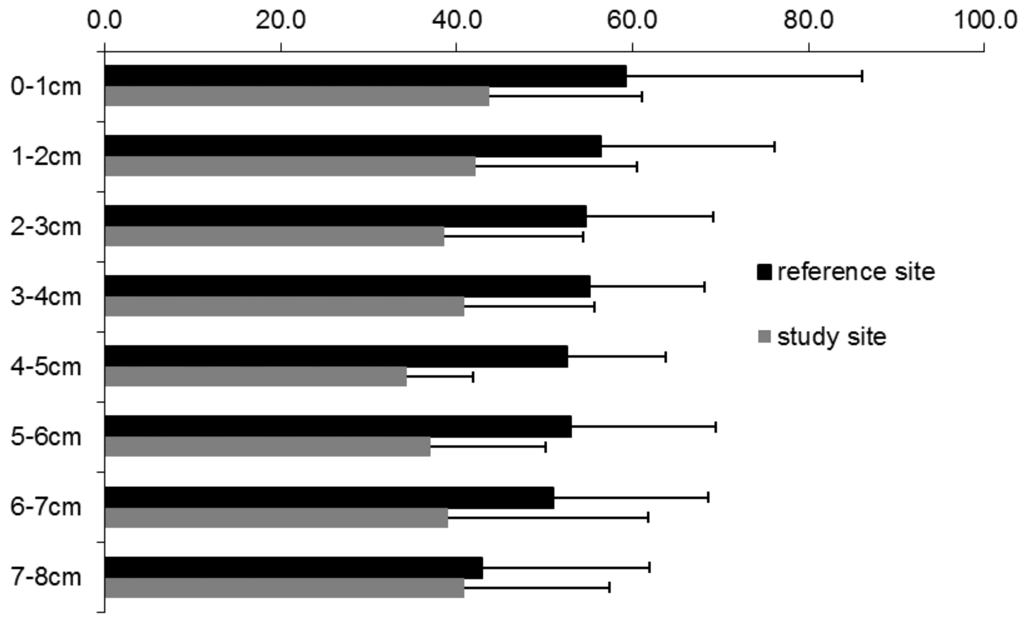


Figure NS8.31 – Average of percentage of totally or partially eaten holes (mean ± standard deviation) of a group of 16 bait lamina in the different depths, for the reference site and study site and exposed during the winter/ spring over 34 days.

Discussion

The exposure period needed to collect the bait lamina was considerably lower in the winter/spring period (34 days) than in the summer/autumn period (90 days). This is most probably due to the higher soil moisture typically found in the winter/spring period that favours the feeding activity of soil through micro and mesofauna. (André et al., 2009; Spehn et al., 2000; Larink e Sommer, 2002). Temperature also plays an important role in feeding activity. Gongalsky et al. (2008) observed an increase in the feeding activity of soil organisms, induced by temperatures of 14 and 24°C. The study site is located in a place with intense solar exposure. In the summer/autumn sampling there was no vegetation in the study site due to land preparation for tree planting, which probably raised the temperature of the soil to very high values, which may have negatively influenced the activity. Apart from that difference, the feeding activity was generally uniform all over the soil profile, independently of the season, which seem to evidence a stable community over the year. In the winter/spring period feeding activity in the reference area tended to be higher if compared to the study site. This observation is in agreement with several studies that report that in areas contaminated with metal(loid)s there is a decrease in feeding activity (André et al., 2009, Boshoff et al., 2014; Filzek et al., 2004, Vorobeichik and Bergman, 2020).Moreover, while within the reference area, the feeding activity tended to decrease with soil depth, in the study area the feeding activity seems more homogeneous through the soil profile, with reduced variation with soil depth. In comparison to the reference site, pitfall traps in the study site showed the highest abundances, in disagreement with the higher feeding activity found in the bait lamina approach of the reference. Microcosm and field studies have shown that macrofauna (i.e earthworms) (Förster et al., 2004; van Gestel et al., 2003), and mesofauna (i.e. enchytraeids are the main feeders on bait lamina. The pitfall trap method is not suitable for collecting such organisms, making difficult a comparison between the results obtained with the two different methods.

• **Chronic earthworm toxicity test**

Materials and methods

Two soil sampling campaigns were carried out in the study site, one in August 2022 (campaign 1) and the other one in January 2023 (campaign 2). A composite soil sample (following the phytoremediation layout by UCP) was collected from the 20 cm top layer of each treatment line (20 samples - L1 to L20). A soil sample was also taken from an adjacent site (unaffected by As and Hg) with similar properties of the study site. This soil sample was used as a reference (REF).
The soil samples were air-dried at room temperature, sieved at 5 mm and defaunated through two freeze–thawing cycles (each cycle comprehending a period of 48h at -20ºC followed by a period of 48h at room temperature). Soil pH (ISO 10390:2005) and water holding capacity (WHC) (ISO 11267:2014) were measured. Each soil composite sample was analysed by ICP-MS for determination of Arsenic and Mercury total concentrations.
Reproduction tests with *Eisenia andrei* followed the procedures described in the ISO guideline 11268-2 (2012). Due to the high number of samples collected in each sampling date, two laboratory tests were performed for each sampling date, using samples L1 to L10 in the first test and samples L11 to L20 in the second test as treatments. The soil sample collected in the reference site was also used as a reference treatment. Additionally, a treatment composed of artificial soil (composed of 5% of air dried *Sphagnum* sp. peat, 20% of kaolinite clay, 74.9% of sand and about 0.1% of CaCO3 to correct the pH to 6.00 ± 0.5) was also used in each test as the control treatment (CT) to confirm the good condition of the test organisms.
Eisenia andrei originated from the Cloverstrategy laboratory cultures were maintained at a constant temperature of 20°C and under a natural photoperiod and used as test organisms in the laboratory tests.
Prior to the deployment of the assays, the moisture of soil samples was adjusted to 50% of the corresponding WHC. Exposures were carried out at 20°C and under a photoperiod of 16 h light and 8 h dark, using 4 replicates per soil sample. Each replicate consisting of a plastic box (7 cm eight, 15 cm length, 8 cm width) containing 500 g of soil (dry weight equivalent). Soil pH and moisture content were measured at the beginning and at the end of the assays. 10 previously washed earthworms, with a fully developed clitellum, more than two months old, and with an individual weight between 250 and 600 mg, were placed in each replicate. The test vessels were covered with a lid to reduce water evaporation. The lid was perforated to create aeration holes. 15 grams fresh weight of cow manure (free of growth promoters, nematicides or similar veterinary pharmaceuticals) previously defaunated and moistened, were added per test container as food at the start of the assay. Thereafter, the adult worms were fed in a similar way weekly during the first 4 weeks of the exposure. At day 28, the surviving adults were removed, counted, washed and weighted to determine the percentage of initial weight. Adults were then left in moistened filter absorbent paper over night to purge their gut. After this period, the earthworms were stored at -20°C for analysis of total body burden concentrations in Mercury and Arsenic according to ISO 17294-2 (ISO 2016) and US EPA Method 200.8 (USEPA 1994), using one composite sample per treatment. At day 56, the number of juveniles hatched was determined in each replicate using a water bath at 50–60 °C for juvenile's recovery from soil. In each sampling date, the earthworm reproduction in soil samples collected in the study site was compared to the earthworms reproduction in soil collected in the reference site by one-way ANOVA to detect significant differences followed by Dunnett's post hoc test to identify the soil samples (i.e. treatments) where significant differences were found. The number of juveniles of each replicate was previously normalized in relation to the average number of juveniles found in the replicates of the CT treatment of the same test.
To detect significant differences between sampling dates a t-test was performed in each treatment line (i.e. L1, L2,…).

Bioaccumulation factors (BAF) was calculated according to the following formula: BAF = Co/Cs, where Co is the metal concentration in earthworms (mg/kg) and Cs is the metal concentration in soil (mg/kg). Pearson's correlation coefficients were calculated comparing in a factorial design soil metal concentrations (As and Hg separately), soil pH, number of juveniles and BAF. All values were previously normalized by LN + 1.

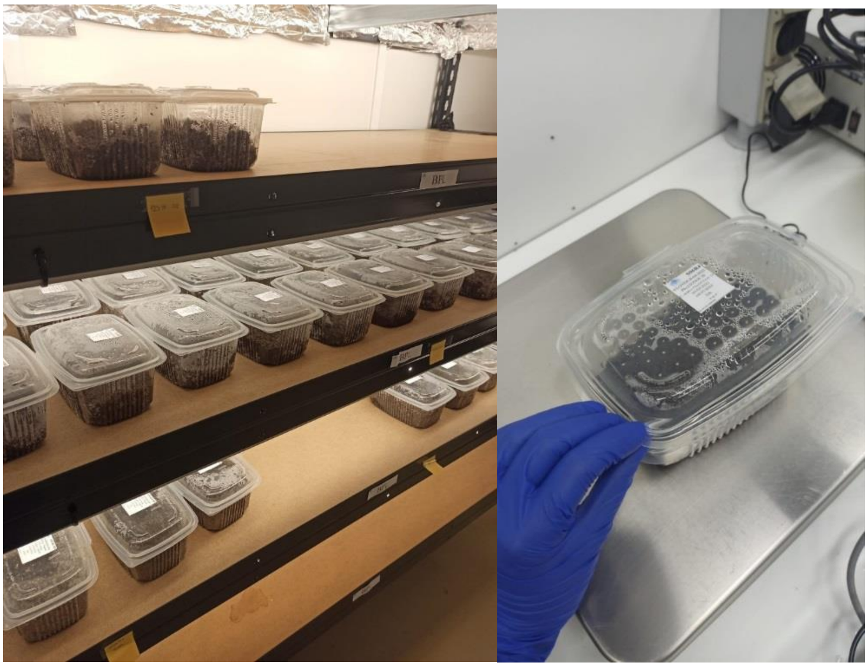


Figure NS8.32 – *Eisenia andrei* reproduction test in the cloverStrategy lab

Results

The average number of juveniles and standard deviation obtained in each test for reference and treatment line are presented in figure NS8.33. Due to the insufficient amount of soil in the first campaign, the L19 sample was not used. In the second campaign, the L12 sample could not be considered in the test, as the soil moisture estimation was not validated. Thus, L19 and L12 were not considered for statistical analysis when comparing reproduction between different sampling dates. Considering the first campaign, there are statistically significant differences to the reference in the treatment lines L4, L5, L6, L9, L14, L16, L17, L18 and L20. In the second campaign, there are statistically significant differences as compared to the reference in the treatment lines L7 and L11. Comparing the reproduction of juveniles in the two campaigns, statistically significant differences between the lines of treatment L5, L6, L7, L8, L10; L11, L14, L15, L16, L17, L18 and L20 were found.

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

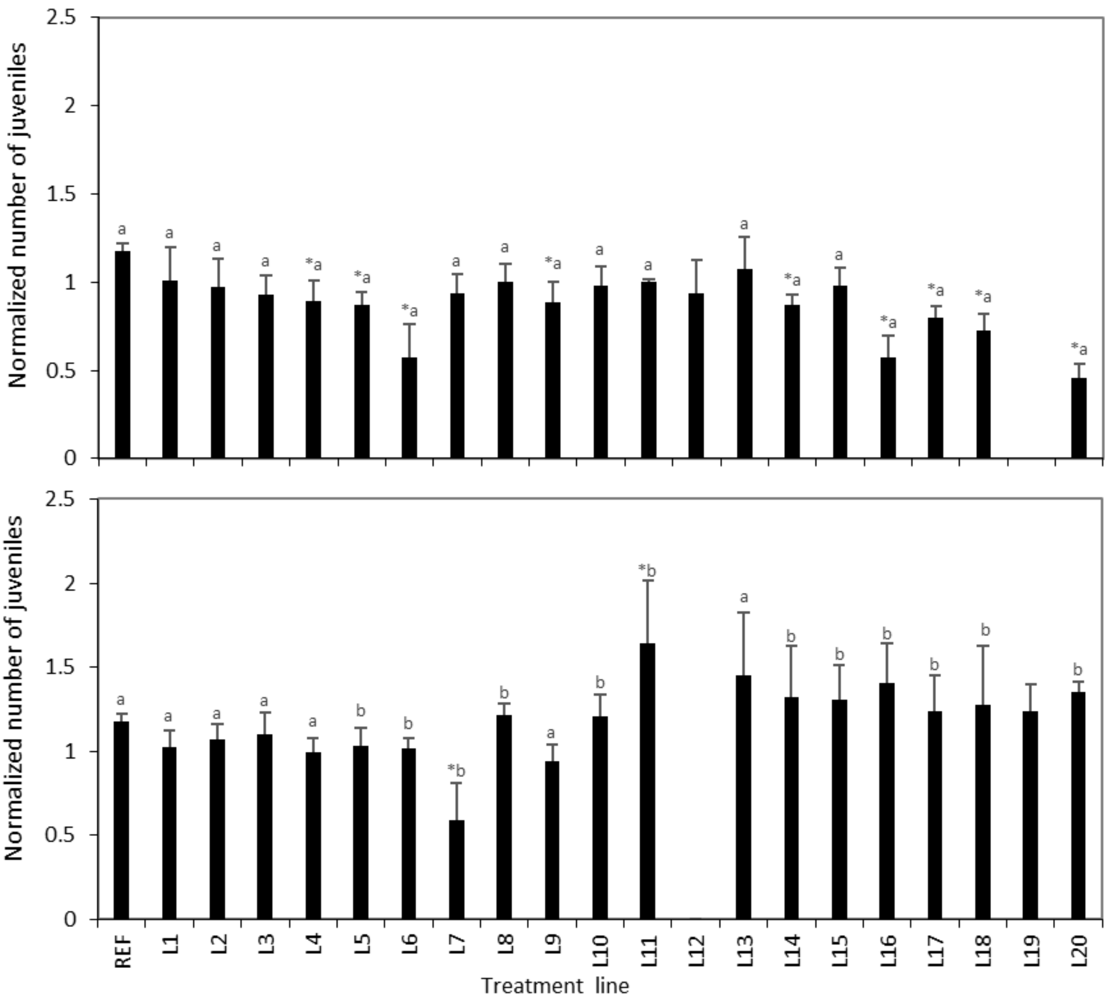


Figure NS8.33 –Number of juveniles (mean ± standard deviation, n=4) normalized (by dividing the number of juveniles of each treatment by the average number of juveniles found in the replicates with artificial soil of the respective tests) in soil samples collected in samplings performed in July 2022 (sampling date 1; first graph) and January 2023 (sampling date 2; second grap). * Significant effect on growth reproduction compared to reference (p≤0.05: Dunnett's Comparison Test); Different letters within the same treatment line and between different sampling dates represent significant differences between sampling 1 and sampling 2 in that treatment line (p≤0.05: t-test);

The concentrations of Hg and As in soil samples (including soil samples from the reference site) of all sampling dates and in the composite earthworm samples of each tested soil and the respective bioaccumulation factors are presented in table NS8.7. There was a decrease of As in soil samples from campaign 1 to campaign 2, except in the treatment lines L8, L13 and L18. Total soil Hg also decreased in most treatment lines in the second campaign except in the treatment lines L3, L4, L13 and L14. The bioaccumulation factors (BAF) calculated for each line of treatment and reference were relatively low especially after the exposure to soil samples of sampling 1. The BAF generally increased in earthworms exposed to soil samples of the second campaign, comparing to those exposed to soil samples of the first campaign.

Table NS8.7 – Total Hg (mg/kg) and As (mg/kg) concentrations in composite soil samples collected in each treatment line (L1 to L20) and in the reference site (REF) and Hg (mg/kg) and As concetration (mg/kg) in the earthworms composite samples after exposure to soil samples and their corresponding bioaccumulation factors (BAF).

Campaign 1							Campaign 2						
	As mg/kg (soil)	Hg mg/kg (soil)	As mg/kg (worm)	Hg mg/kg (worm)	BAF As	BAF Hg		As mg/kg (soil)	Hg mg/kg (soil)	As mg/kg (worm)	Hg mg/kg (worm)	BAF As	BAF Hg
							REF	10.50	<0.20	8.46	0.02	0.81	-
L1	33.70	2.39	12.60	0.02	0.37	0.009	**L1**	23.10	1.54	10.20	0.07	0.44	0.043
L2	78.40	13.10	17.50	0.31	0.22	0.024	**L2**	46.60	5.47	13.90	0.22	0.30	0.039
L3	69.50	8.73	19.10	0.24	0.27	0.028	**L3**	58.00	15.50	22.00	0.52	0.38	0.033
L4	95.80	10.10	19.40	0.30	0.20	0.030	**L4**	58.20	13.20	22.00	0.40	0.38	0.030
L5	109.00	10.30	23.50	0.23	0.22	0.022	**L5**	78.40	9.55	34.10	0.39	0.43	0.041
L6	160.00	17.10	35.00	0.54	0.22	0.032	**L6**	54.50	9.65	26.20	0.30	0.48	0.031
L7	138.00	20.80	30.80	0.29	0.22	0.014	**L7**	63.10	9.12	31.00	0.48	0.49	0.053
L8	51.70	48.00	15.30	0.11	0.30	0.002	**L8**	51.90	11.20	20.40	0.19	0.39	0.017
L9	71.10	10.00	19.30	0.36	0.27	0.036	**L9**	63.90	9.19	27.40	0.44	0.43	0.048
L10	79.20	9.76	17.60	0.20	0.22	0.020	**L10**	59.60	8.26	26.40	0.28	0.44	0.034
L11	32.40	3.28	10.10	0.15	0.31	0.046	**L11**	24.70	1.85	8.60	0.08	0.35	0.041
L13	9.23	0.66	3.22	0.04	0.35	0.057	**L13**	15.30	0.69	4.83	0.05	0.32	0.070
L14	14.90	0.57	2.71	0.03	0.18	0.049	**L14**	12.80	0.99	3.22	0.09	0.25	0.091
L15	21.60	0.98	3.72	0.05	0.17	0.048	**L15**	6.55	0.26	2.13	0.04	0.33	0.147
L16	24.80	0.83	3.23	0.03	0.13	0.040	**L16**	14.90	0.74	3.11	0.06	0.21	0.081
L17	21.60	0.81	3.74	0.04	0.17	0.053	**L17**	17.90	0.71	3.13	0.05	0.17	0.075
L18	13.70	0.48	3.00	0.03	0.22	0.065	**L18**	16.00	0.52	3.28	0.07	0.21	0.126
L20	18.60	0.28	3.50	0.03	0.19	0.116	**L20**	16.20	0.85	2.66	0.06	0.16	0.075

Pearson's correlation coefficients calculated (table NS8.9) showed significant positive correlation between soil As and soil Hg concentrations, Mercury and As concentrations in soil and the soil pH, and a significant negative correlation (although with a lower correlation coefficient) between BAF values of Hg and As.

Table NS8.9 – Pearson+s correlation coefficient matrix between total soil As and Hg, soil pH; BAF of arsenic (As) and mercury (Hg) - * - Means a significant correlation coefficient, p ≤ 0.05)

	Number of juveniles	Arsenic (mg/kg)	Mercury (mg/kg)	pH	BAF (As)
Arsenic (mg/kg)	0.16				
Mercury (mg/kg)	0.29	**0.88***			
pH	0.30	**0.80***	**0.92***		
BAF (As)	0.21	0.16	0.06	0.07	
BAF (Hg)	-0.19	-0.41	-0.37	-0.37	**-0.65***

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

Discussion

The results of reproduction of *Eisenia andrei* show that in the first campaign there was a statistically different effect in relation to the reference in a greater number of treatment lines when compared to the second campaign. Although samples from the first campaign have higher concentrations in total As and Hg, the differences would not be due to the concentration of As and Hg since there is no correlation between the metal(loid)s and the number of juveniles. The reference used has a higher As concentration than some treatment lines. A reference with a lower amount of As in the soil should be used for better comparisons. Between the two sampling dates, there is are significant differences in most of the treatment lines, indicating probable changes the soil properties will change between sampling times. An evaluation of other variables would be necessary for a deeper analysis of the results. Although there was a decrease in total soil Hg and As between the two campaigns, the bioaccumulation factors increased slightly indicating that those elements might have become more bioavailable. This explanation is congruent with the perceived increased mobility of metals as discussed in 3.4. Chemical speciation of these 2 metals can shade light over those phenomena.

There is a negative correlation between BAF-values for Hg and As, result that could indicate that some factors can determine a preferential bioavailability or bioaccumulation of each metal(loid). Further investigation is required.

Conclusions

The monitoring tools used for this approach have proved themselves useful, sensitive and complementary.

Regarding the results of the phytoremediation techniques, and although its period has been short, and experimental data has not yet been fully explored, so effects of specific treatments cannot be fully analyzed, nor the influence of the groundwater regime nor other environmental variables can be accounted, the experimental site has experienced notable reductions in the field-average concentration of some elements, indicating a mobilization of some contaminants, notably, As, Ba, Pb, and Hg. Results also show an increase in bioaccumulation indexes after phytoremediation; such results indicate an increase in mobility and in bioavailability, while exhibiting lower effect on the reproduction of earthworms. These results indicate some effective soil remediation that the site became more amenable to other complementary remediative techniques as well. Further assessment of the transfer of the major contaminants to different compartments (groundwater, atmosphere and bioaccumulation) should be monitored in subsequent monitoring campaigns over an extended period of phytoremediation.

Chemical speciation of the major contaminants of concern (As and Hg), detailed texture analysis, groundwater monitoring and field-lysimeters may become instrumental to an enhanced interpretation of the reduction of their concentrations in soil.

Further works should ensure the continuity of the monitoring of feeding activity, abundance and diversity of soil organisms, as indicators of the ecological recovery of similar impacted sites.

References

AGUIAR, C. & SERRANO, A., 2013. *Catalogo e atlas dos coleopteros carabideos (Coleoptera, Carabidae) de Portugal continental*. Lisboa: Sociedade Portuguesa de Entomologia. 265pp.

ANDRÉ, A., ANTUNES, S.C., GONÇALVES, F., PEREIRA, R., 2009. Bait–lamina assay as a tool to assess the effects of metal contamination in the feeding activity of soil invertebrates within a uranium mine area. Environ. Pollut. 157, 2368–2377.

BOSHOFF, M., DE JONGE, M., DARDENNE, F., BLUST, R., BERVOETS, L., 2014. The impact of metal pollution on soil faunal and microbial activity in two grassland ecosystems. Environ. Res. 134, 169–180

BUTOVSKY, R.O., 1994. Carabids in roadside ecosystems: perspectives of bioindication, in: Desender, K., M.Dufrene, M.Loreau, M.L.Luff, J.P.Maelfait (Eds.), *Carabid Beetles: Ecology and Evolution*. Kluwer Academic Press, Dordrecht, 241–246.

BUTOVSKY, R.O., 2011. Heavy metals in carabids (Coleoptera, Carabidae). *Zookeys*, 222: 215–22.

DAVIS, M.R.H., ZHAO, F., MCGRATH, S.P., 2004. Pollution–induced community tolerance of soil microbes in response to a zinc gradient. Environ. Toxicol. Chem. 23, 2665–2672.

FILZEK, P.D.B., SPURGEON, D.J., BROLL, G., SVENDSEN, C., HANKARD, KAMMENGA, J.E., WEEKS, J.M., 2004A. Pedological characteristics of sites along a transect from a primary cadmium/lead/zinc smelting works. Ecotoxicology 13, 725–737.

FILZEK, P.D.B., SPURGEON, D.J., BROLL, SVENDSEN, G, HANKARD, P.K., PAREKH, N., STUBBERUD, H.E., WEEKS, J.M., 2004. Metal effects on soil invertebrate feeding: measurements using the bait lamina method. Ecotoxicology 13, 807–816.

GONGALSKY, K.B., PERSSON, T., POKARZHEVSKII, A.D., 2008. Effects of soil temperature and moisture on the feeding activity of soil animals as determined by the bait lamina test. Applied Soil Ecol. 39, 84–90.

ISO (International Organization for Standardization) (2005) Soil quality — Determination of pH. ISO 10390:2005. Geneva, Switzerland.

ISO (International Organization for Standardization) (2012) Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei. ISO 11268-2:2012. Geneva, Switzerland.

ISO (International Organization for Standardization) (2014) Soil quality – Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants. ISO 11267:2014. Geneva, Switzerland.

ISO (International Organization for Standardization) (2016) Water quality — Application of inductively coupled plasma mass spectrometry (ICP-MS) — Part 2: Determination of selected elements including uranium isotopes ISO 17294-2:2016. Geneva, Switzerland

KRUSE, P.D., TOFT, S. & SUNDERLAND, K.D., 2008. Temperature and prey capture: opposite relationships in two predator taxa. *Ecological Entomology*, 33: 305–312.

OLIVEIRA, A., 2006. Os carabídeos (Coleoptera, Carabidae) da serra de monchique (Sítio Rede Natura 2000): biodiversidade e ecologia. Tese de doutoramento. Évora, Portugal.

ORDENS, CARLOS., 2007. Estudo da Contaminação do aquífero superior na região de Estarreja. Tese de mestrado. Coimbra, Portugal : Universidade de Coimbra, 2007.

RIBEIRO ET AL.., 1979 – *Introduction à la Géologie Générale du Portugal*. Serv. Geol. Portugal, pp. 114, Lisboa.

SERRANO, J., LENCINA, J.L. & ANDÚJAR, A., 2003. Distribution patterns of iberian Carabidae (Insecta, Coleoptera). *Graellsia* 59: 129–153.

U.S. EPA. (1994). “Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry,” Revision 5.4. Cincinnati, OH.

VÉLE, A., HOLUŠA, J., & FROUZ, J. (2009). Ecological requirements of some ant species of the genus Formica (Hymenoptera, Formicidae) in spruce forests. Journal of Forest Science, 55(No. 1), 32–40.

VOROBEICHIK, E.L., BERGMAN, I.E., 2020. Bait-lamina test in the assessment of polluted soils: choice of exposure duration. Russ. J. Ecol. 51 (5), 430–439.

Appendices 1 - soil chemical analysis (study site – July 2022)

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Characteristics																					
Dry matter	% (w/w)	96.8	92.5	91.5	92.8	95.2	93.5	94.2	93.7	90.3	92.0	94.5	91.5	93.7	93.0	94.9	90.9	91.4	91.3	94.1	91.9
Total Organic Carbon (TOC)	g/kg ms	17	14	16	17	11	18	12	11	21	19	13	14	21	16	14	18	14	13	12	13
Elements																					
Arsenic (As)	mg/kg dm	55	38	30	66	51	46	190	18	41	63	79	11	25	23	17	31	18	17	11	18
Barium (Ba)	mg/kg dm	92	59	28	76	78	61	340	22	46	87	100	<15	31	29	21	35	21	24	17	27
Beryllium (Be)	mg/kg dm	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Calcium oxide (CaO), total (calc.)	mg/kg dm	4000	1500	2100	3300	3300	61	12000	1800	<50	5000	5100	520	1400	1100	800	2800	570	880	640	910
Cadmium (Cd)	mg/kg dm	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	0.42	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40
Cobalt (Co)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Chromium (Cr)	mg/kg dm	<5.0	5.5	<5.0	5.9	7.0	5.2	<5.0	<5.0	<5.0	<5.0	6.0	<5.0	5.3	6.3	5.2	8.4	<5.0	6.6	5.9	6.0
Copper (Cu)	mg/kg dm	13	12	10	12	15	12	21	5.7	15	16	22	9.3	14	15	14	22	15	16	12	16
Mercury (Hg)	mg/kg dm	22	3.1	2.2	6.3	10	4.0	23	1.9	6.4	8.9	9.9	0.56	2.7	1.6	0.71	1.2	1.2	0.73	0.29	1.0
Potassium oxide (K_2O)	g/kg dm	0.46	0.59	0.50	0.47	0.52	0.69	0.36	0.24	0.44	0.33	0.44	0.29	0.55	0.55	0.38	0.63	0.37	0.53	0.50	0.51
Magnesium as MgO	mg/kg dm	870	1100	970	980	1000	1500	700	730	860	630	910	520	1000	1200	960	1600	760	1200	1100	1100
Molybdenum (Mo)	mg/kg dm	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
ICP-MS Sodium (Na_2O)	mg/kg dm	110	160	140	140	150	<50	100	52	<50	68	100		100	110	65	150	56	100	150	94
Nickel (Ni)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	5.1	<5.0	6.2	<5.0	<5.0	<5.0	<5.0
Phosphorus	g/kg dm	0.14	0.17	0.16	0.15	0.17	<0.050	0.16	0.087	<0.050	0.13	0.23	0.13	0.19	0.24	0.14	0.25	0.14	0.16	0.12	0.14
Phosphorus total (PO_4)	g/kg dm	0.42	0.52	0.48	0.46	0.53	<0.15	0.50	0.27	<0.15	0.41	0.70	0.41	0.60	0.73	0.44	0.76	0.42	0.49	0.37	0.43
Phosphorus (as P_2O_5)	g/kg dm	0.31	0.39	0.36	0.35	0.39	<0.12	0.38	0.20	<0.12	0.31	0.53	0.31	0.45	0.55	0.33	0.57	0.31	0.36	0.27	0.32
Lead (Pb)	mg/kg dm	95	66	40	130	110	79	340	30	65	130	160	20	41	48	27	53	30	32	22	32
Sulphur (S)	g/kg dm	0.22	0.26	0.28	0.27	<0.20	<0.20	0.41	<0.20	<0.20	0.22	0.35	<0.20	0.41	0.36	0.26	0.40	<0.20	0.22	0.22	0.36
Sulphur as sulphate (SO_4)	g/kg dm	0.66	0.77	0.85	0.82	<0.60	<0.60	1.2	<0.60	<0.60	0.67	1.0	<0.60	1.2	1.1	0.78	1.2	<0.60	0.66	0.66	1.1

Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Antimony (Sb)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	4.8	<2.0	<2.0	2.1	2.4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Selenium (Se)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	4.7	<2.0	<2.0	2.1	2.2	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Tin (Sn)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Vanadium (V)	mg/kg dm	6.2	8.2	6.8	6.9	7.7	8.9	5.5	<5.0	8.1	5.7	7.8	<5.0	8.5	10	6.9	11	6.0	8.2	6.7	7.6
Zinc (Zn)	mg/kg dm	81	59	47	72	95	73	140	32	75	86	160	52	82	110	97	140	73	110	92	95
Mono Aromatic Hydrocarbons																					
Benzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Toluene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Ethylbenzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
o-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
m,p-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Xylenes (sum)	mg/kg dm	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
BTEX (sum)	mg/kg dm	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
TPH Aliphatic Aromatic split																					
Aliphatics >C5 - C6	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C6 - C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C8 - C10	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Total Vol. Aliphatics	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Aromatics >C6-C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics >C8 - C10	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Total Vol. Aromatics	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	14	<12	<12	<12	17	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
Total Aliphatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
Aromatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Aromatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aromatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
Total Aromatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
EPH (C10-C35)	mg/kg dm	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Polycyclic Aromatic Hydrocarbons, PAH																					
Naphthalene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthylene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluorene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phenanthrene	mg/kg dm	0.043	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	0.019	<0.010	<0.010	<0.010	<0.010	<0.010
Anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluoranthene	mg/kg dm	0.066	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.042	<0.010	<0.010	<0.010	0.027	<0.010	0.014	<0.010	0.011	<0.010	<0.010	<0.010
Pyrene	mg/kg dm	0.056	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.036	<0.010	<0.010	<0.010	0.023	<0.010	0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benz(a)anthracene	mg/kg dm	0.031	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010	0.016	<0.010	0.012	<0.010	<0.010	<0.010	<0.010	<0.010
Chrysene	mg/kg dm	0.031	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.025	<0.010	<0.010	<0.010	0.018	<0.010	0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(b)fluoranthene	mg/kg dm	0.034	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.032	<0.010	<0.010	<0.010	0.021	<0.010	0.010	<0.010	0.013	<0.010	<0.010	<0.010
Benzo(k)fluoranthene	mg/kg dm	0.014	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(a)pyrene	mg/kg dm	0.029	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.024	<0.010	<0.010	<0.010	0.016	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Dibenz(a,h)anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(g,h,i)perylene	mg/kg dm	0.021	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.021	<0.010	<0.010	<0.010	0.013	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Indeno(1,2,3-cd)pyrene	mg/kg dm	0.016	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
PAH 10 VROM (sum)	mg/kg dm	0.25	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.17	<0.10	<0.10	<0.10	0.11	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PAH 16 EPA (sum)	mg/kg dm	0.34	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	0.24	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16
Physical and chemical analyses																					
Measuring temperature (pH-H_2O)	°C	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Acidity (pH-H_20)		7.4	6.1	5.6	7.2	6.9	6.8	7.8	6.7	6.1	7.6	7.4	5.3	5.6	5.3	5.4	5.2	5.4	5.2	5.3	5.4
Kjeldahl Nitrogen (N)	mg/kg dm																				

Appendices 2 - soil chemical analysis (study site – March 2023)

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Characteristics																					
Dry matter	% (w/w)	71.5	78.3	76.0	78.0	82.2	83.1	80.1	86.7	85.4	82.7	83.7	84.9	84.6	89.1	83	83.4	86.8	82.4	82.9	84.7
Total Organic Carbon (TOC)	g/kg ms	26	20	22	16	20	21	15	14	22	16	10	11	17	12	13	11	15	15	11	8.6
Elements																					
Arsenic (As)	mg/kg dm	39	27	22	82	53	33	67	27	48	88	28	13	17	6.4	24	26	19	16	13	14
Barium (Ba)	mg/kg dm	73	47	25	74	56	43	74	62	50	120	33	18	17	<15	28	27	32	24	18	21
Beryllium (Be)	mg/kg dm	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Calcium oxide (CaO), total (calc.)	mg/kg dm	1900	990	810	2500	1900	1600	3700	1500	1800	7200	3000	550	550	350	740	670	600	630	480	500
Cadmium (Cd)	mg/kg dm	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40
Cobalt (Co)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Chromium (Cr)	mg/kg dm	5.8	5.4	<5.0	5.7	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	5.7	5.9	5.3	6.2	5.1	5.1
Copper (Cu)	mg/kg dm	15	12	11	16	14	12	15	9.3	15	18	17	13	14	5.6	15	17	15	18	13	15
Mercury (Hg)	mg/kg dm	8.9	1.8	1.2	6.2	6.7	3.5	7.7	2.5	6.7	14	3.2	0.57	0.74	0.35	0.93	0.76	0.86	0.52	0.23	0.32
Potassium oxide (K2O)	g/kg ms	0.69	0.60	0.55	0.69	0.51	0.49	0.4	0.29	0.42	0.33	0.37	0.39	0.46	0.27	0.46	0.42	0.7	0.52	0.51	0.54
Magnesium as MgO	mg/kg dm	1300	1100	1100	1300	990	850	750	540	770	710	710	700	810	480	960	870	1300	1200	1100	1100
Molybdenum (Mo)	mg/kg dm	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
ICP-MS Sodium (Na2O)	mg/kg dm	200	220	230	140	71	180	220	120	210	170	140	140	140	130	200	180	230	160	220	180
Nickel (Ni)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Phosphorus	g/kg ms	0.19	0.19	0.15	0.21	0.13	0.16	0.17	0.11	0.16	0.16	0.13	0.17	0.18	0.1	0.17	0.18	0.18	0.18	0.14	0.14
Phosphorus total (PO4)	g/kg ms	4.8	6.2	3.7	4.8	4.3	0.49	3.1	3	5.5	0.48	0.41	0.51	0.55	0.31	4.4	0.53	0.56	5.4	4.6	5
Phosphorus (as P2O5)	g/kg ms	3.5	4.6	2.8	3.6	3.2	0.36	2.3	2.3	4.1	0.36	0.31	0.38	0.41	0.23	3.3	0.39	0.42	4.1	3.4	3.7
Lead (Pb)	mg/kg dm	66	46	32	100	97	68	120	60	70	210	54	27	28	12	35	38	30	29	21	21
Sulphur (S)	g/kg ms	0.33	0.29	<0.20	0.25	<0.20	<0.20	0.3	<0.20	0.26	<0.20	0.22	<0.20	<0.20	<0.20	0.23	0.2	<0.20	<0.20	<0.20	<0.20
Sulphur as sulphate (SO4)	g/kg ms	1.00	0.88	<0.60	0.75	<0.60	<0.60	0.89	<0.60	0.78	<0.60	0.67	<0.60	<0.60	<0.60	0.68	0.6	<0.60	<0.60	<0.60	<0.60
Antimony (Sb)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.8	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Selenium (Se)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.9	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Tin (Sn)	mg/kg dm	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Vanadium (V)	mg/kg dm	9.3	9.8	8.0	9.4	7.2	6.6	6.6	5	7.4	5.8	6.4	6.7	7.9	<5.0	8.3	11	8.8	7.8	7.1	6.6
Zinc (Zn)	mg/kg dm	80	67	51	95	83	66	84	58	72	110	120	78	73	38	110	110	84	110	99	84
Mono Aromatic Hydrocarbons																					
Benzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Toluene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Ethylbenzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
o-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
m,p-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Xylenes (sum)	mg/kg dm	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
BTEX (sum)	mg/kg dm	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
TPH Aliphatic Aromatic split																					
Aliphatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.8	<2.0	3.1	2.6	3	2.3	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
Total Aliphatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
Aromatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.1	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aromatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aromatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
Total Aromatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
EPH (C10-C35)	mg/kg dm	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Aliphatics >C5 - C6	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C6 - C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C8 - C10	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.3	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Total Vol. Aliphatics	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Aromatics sum C6-C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics sum C8-C10	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Total Vol. Aromatics	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0

	Units	L1C1	L2C2	L3C3	L4C2	L5C1	L6C2	L7C3	L8C2	L9C1	L10C2	L11C3	L12C2	L13C1	L14C2	L15C3	LC16C2	L17C1	L18C2	L19C3	L20C2
Polycyclic Aromatic Hydrocarbons, PAH																					
Naphthalene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthylene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluorene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phenanthrene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluoranthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.012	<0.010
Pyrene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010
Benz(a)anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.014	<0.010
Chrysene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.016	<0.010
Benzo(b)fluoranthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.01	<0.010	<0.010	<0.010	<0.010	0.011	<0.010	<0.010	0.02	<0.010
Benzo(k)fluoranthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(a)pyrene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.016	<0.010
Dibenz(a,h)anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(g,h,i)perylene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.011	<0.010
Indeno(1,2,3-cd)pyrene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.013	<0.010
PAH 10 VROM (sum)	mg/kg dm	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PAH 16 EPA (sum)	mg/kg dm	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16
Physical and chemical analyses																					
Measuring temperature (pH-H2O)	°C	19	19	19	19	19	20	19	19	19	19	19	20	19	19	19	19	19	19	19	19
Acidity (pH-H20)		6.5	6.3	6.3	6.5	7	6.9	7.2	6.6	6.7	7.9	7.2	5.7	5.7	5.7	5.7	5.5	5.4	5.6	5.9	5.6
Kjeldahl Nitrogen (N)	g/kg ms	1.6	1.2	1.2	1.9	1.2	1.2	1.4	0.7	1.2	1.2	1.1	1.2	1.5	0.7	1.2	1.1	1.1	1.2	0.9	0.8

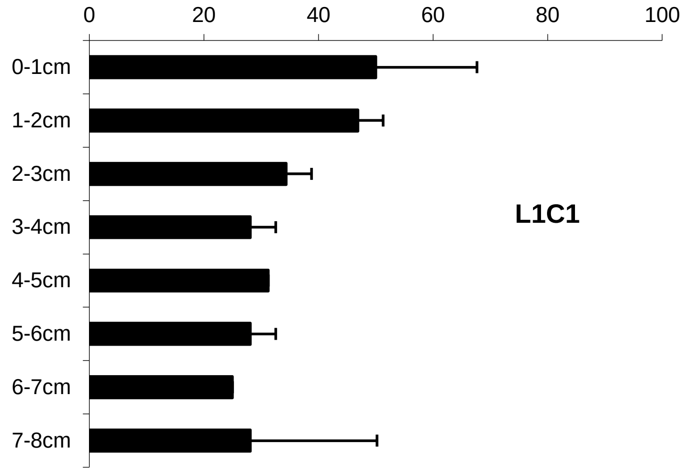
Appendices 3 - soil chemical analysis (Pitfall traps and bait lamina reference area - March 2023)

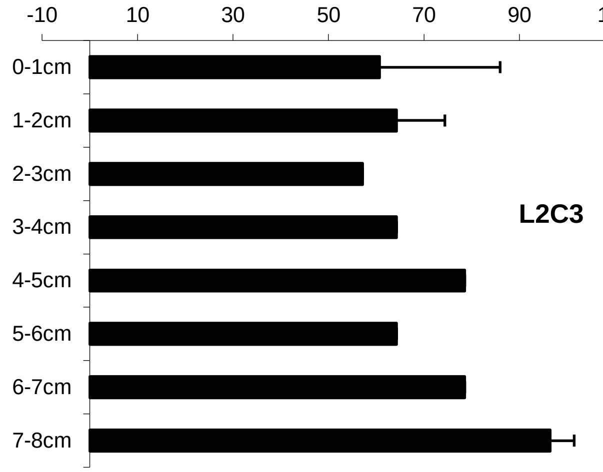
	Units	REF1	REF2	REF3	REF4	REF5	REF6	REF7
Characteristics								
Dry matter	% (w/w)	82	80.7	80.7	82	79.4	85.4	93.9
Total Organic Carbon (TOC)	g/kg ms	17	17	20	18	23	16	6.2
Elements								
Arsenic (As)	mg/kg dm	9.4	8.3	6.5	8.6	6	15	16
Barium (Ba)	mg/kg dm	120	140	100	140	160	59	22
Beryllium (Be)	mg/kg dm	1.5	1.9	1	1.9	1.6	<1.0	<1.0
Calcium oxide (CaO), total (calc.)	mg/kg dm	920	600	650	690	1000	540	400
Cadmium (Cd)	mg/kg dm	<0.40	<0.40	<0.40	<0.40	<0.40	<0.4	<0.40
Cobalt (Co)	mg/kg dm	6.5	9.3	5.8	7.5	7.6	<5.0	<5.0
Chromium (Cr)	mg/kg dm	14	15	11	14	12	6.8	<5.0
Copper (Cu)	mg/kg dm	11	8.2	7.7	7.4	7.2	9	5.8
Mercury (Hg)	mg/kg dm	0.34	<0.10	<0.10	0.16	<0.10	0.43	0.23
Potassium oxide (K2O)	g/kg ms	3.2	3.3	2.5	3.7	3.6	1.5	0.59
Magnesium as MgO	mg/kg dm	5100	5500	4200	6300	6000	2700	980
Molybdenum (Mo)	mg/kg dm	<1.5	<1.5	<1.5	<1.5	<1.5	<1.6	<1.5
ICP-MS Sodium (Na2O)	mg/kg dm	200	220	150	250	200	100	63
Nickel (Ni)	mg/kg dm	7.5	8.1	5.8	7.7	6.9	<5.0	<5.0
Phosphorus	g/kg ms	0.28	0.3	0.25	0.28	0.29	0.2	0.1
Phosphorus total (PO4)	g/kg ms	0.82	0.84	6.6	0.84	0.84	0.63	0.3
Phosphorus (as P2O5)	g/kg ms	0.61	0.63	4.9	0.63	0.63	0.47	0.22
Lead (Pb)	mg/kg dm	28	24	22	26	39	24	<10
Sulphur (S)	g/kg ms	0.21	<0.20	<0.20	<0.20	<0.20	0.2	<0.20
Sulphur as sulphate (SO4)	g/kg ms	0.62	<0.60	<0.60	<0.60	<0.60	0.6	<0.60
Antimony (Sb)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

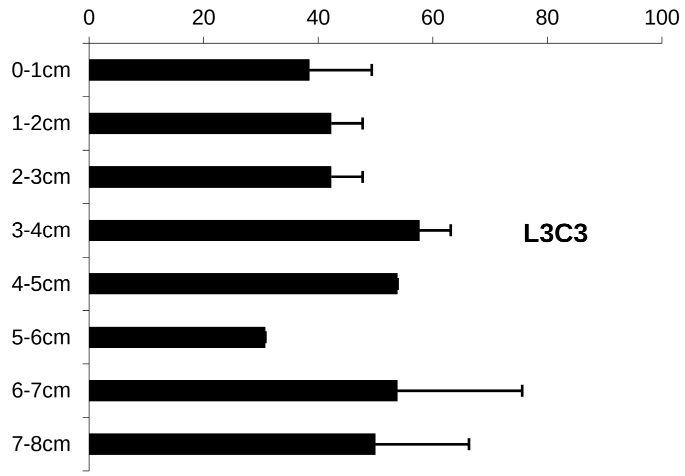
	Units	REF1	REF2	REF3	REF4	REF5	REF6	REF7
Selenium (Se)	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Tin (Sn)	mg/kg dm	15	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
Vanadium (V)	mg/kg dm	31	36	26	35	30	15	5.7
Zinc (Zn)	mg/kg dm	52	51	44	46	57	33	30
Mono Aromatic Hydrocarbons								
Benzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Toluene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Ethylbenzene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
o-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
m,p-Xylene	mg/kg dm	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Xylenes (sum)	mg/kg dm	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
BTEX (sum)	mg/kg dm	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
TPH Aliphatic Aromatic split								
Aliphatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aliphatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	<12	<12	<12
Total Aliphatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
Aromatics >C10 - C12	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics >C12 - C16	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aromatics >C16 - C21	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Aromatics >C21 - C35	mg/kg dm	<12	<12	<12	<12	<12	<12	<12
Total Aromatics (C10-C35)	mg/kg dm	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
EPH (C10-C35)	mg/kg dm	<50	<50	<50	<50	<50	<50	<50
Aliphatics >C5 - C6	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C6 - C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aliphatics >C8 - C10	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Total Vol. Aliphatics	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
Aromatics sum C6-C8	mg/kg dm	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Aromatics sum C8-C10	mg/kg dm	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0

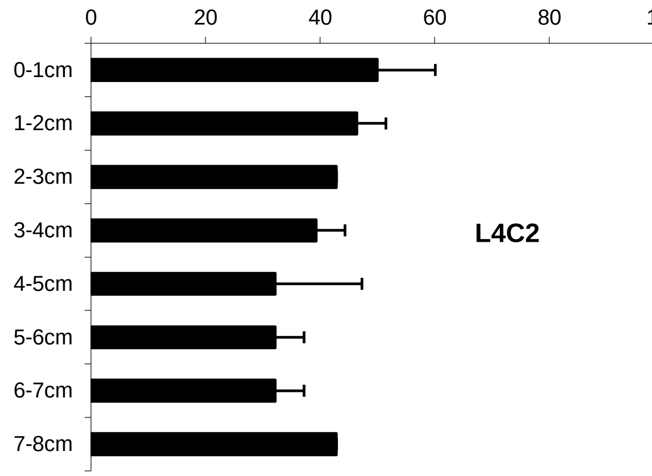
	Units	REF1	REF2	REF3	REF4	REF5	REF6	REF7
Total Vol. Aromatics	mg/kg dm	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0	<8.0
Polycyclic Aromatic Hydrocarbons, PAH								
Naphthalene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthylene	mg/kg dm	0.015	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Acenaphthene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluorene	mg/kg dm	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phenanthrene	mg/kg dm	0.13	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Anthracene	mg/kg dm	0.041	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Fluoranthene	mg/kg dm	0.15	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Pyrene	mg/kg dm	0.11	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benz(a)anthracene	mg/kg dm	0.087	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Chrysene	mg/kg dm	0.076	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(b)fluoranthene	mg/kg dm	0.081	<0.010	0.015	<0.010	<0.010	<0.010	<0.010
Benzo(k)fluoranthene	mg/kg dm	0.033	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(a)pyrene	mg/kg dm	0.061	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Dibenz(a,h)anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Benzo(g,h,i)perylene	mg/kg dm	0.026	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Indeno(1,2,3-cd)pyrene	mg/kg dm	0.034	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
PAH 10 VROM (sum)	mg/kg dm	0.64	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
PAH 16 EPA (sum)	mg/kg dm	0.86	<0.16	<0.16	<0.16	<0.16	<0.16	<0.16
Physical and chemical analyses								
Measuring temperature (pH-H2O)	°C	19	19	19	19	19	19	19
Acidity (pH-H20)		5.6	5.5	5.8	5.5	5.7	5.5	5.8
Kjeldahl Nitrogen (N)	g/kg ms	1.6	1.3	1.3	1.2	1.2	1.1	0.4

Appendices 4 – Total bait lamina record

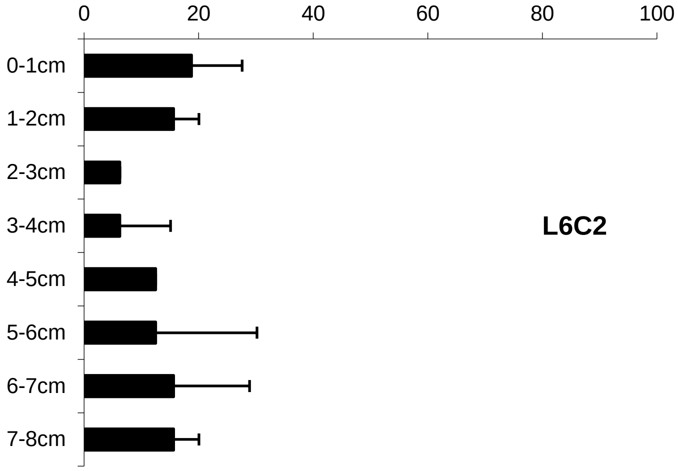


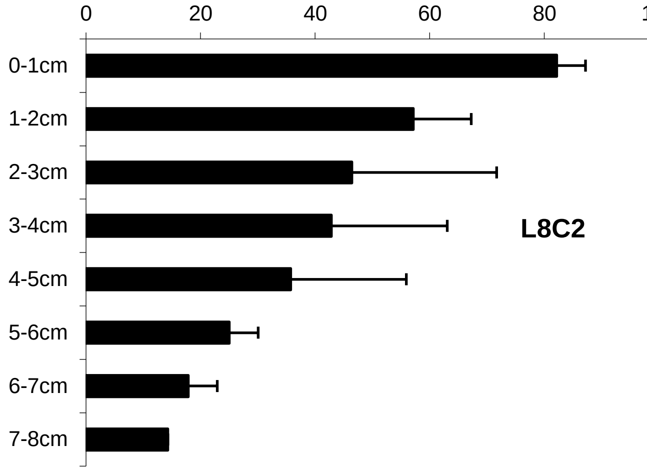


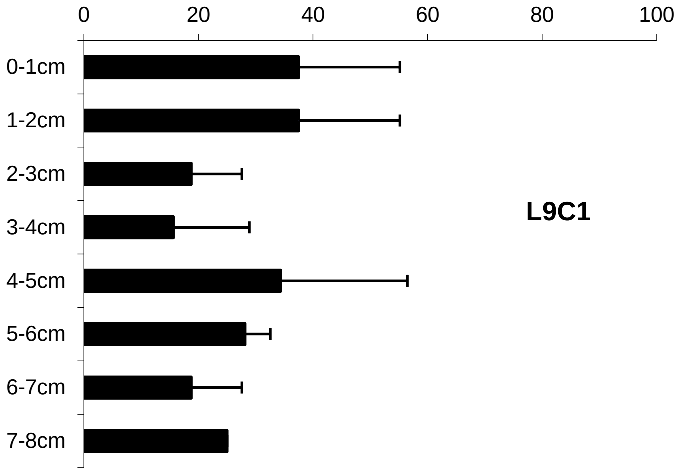


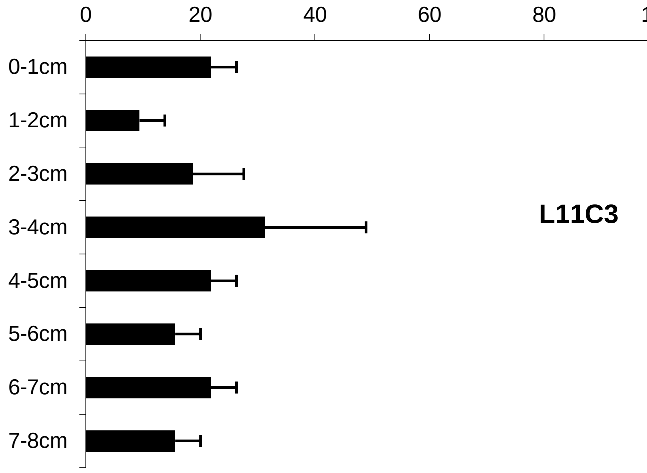


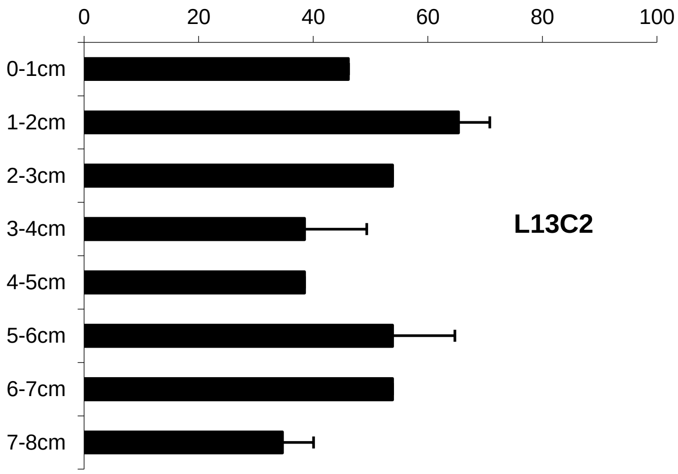
Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

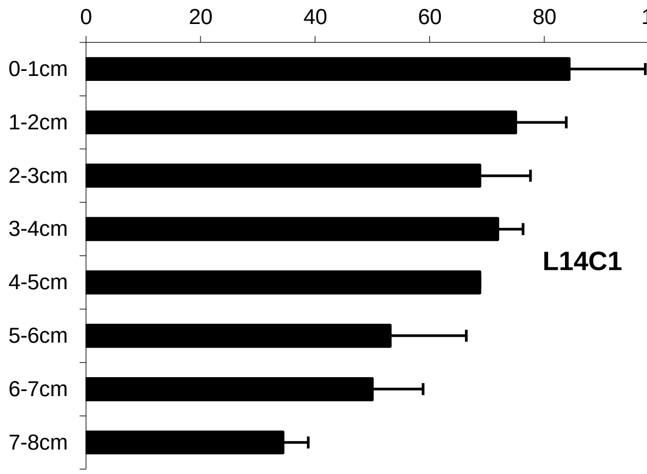

0
20
40
60
80
100
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L6C2


0
20
40
60
80
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L8C2


0
20
40
60
80
100
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L9C1


0
20
40
60
80
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L11C3


0
20
40
60
80
100
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L13C2


0
20
40
60
80
0-1cm
1-2cm
2-3cm
3-4cm
4-5cm
5-6cm
6-7cm
7-8cm
L14C1

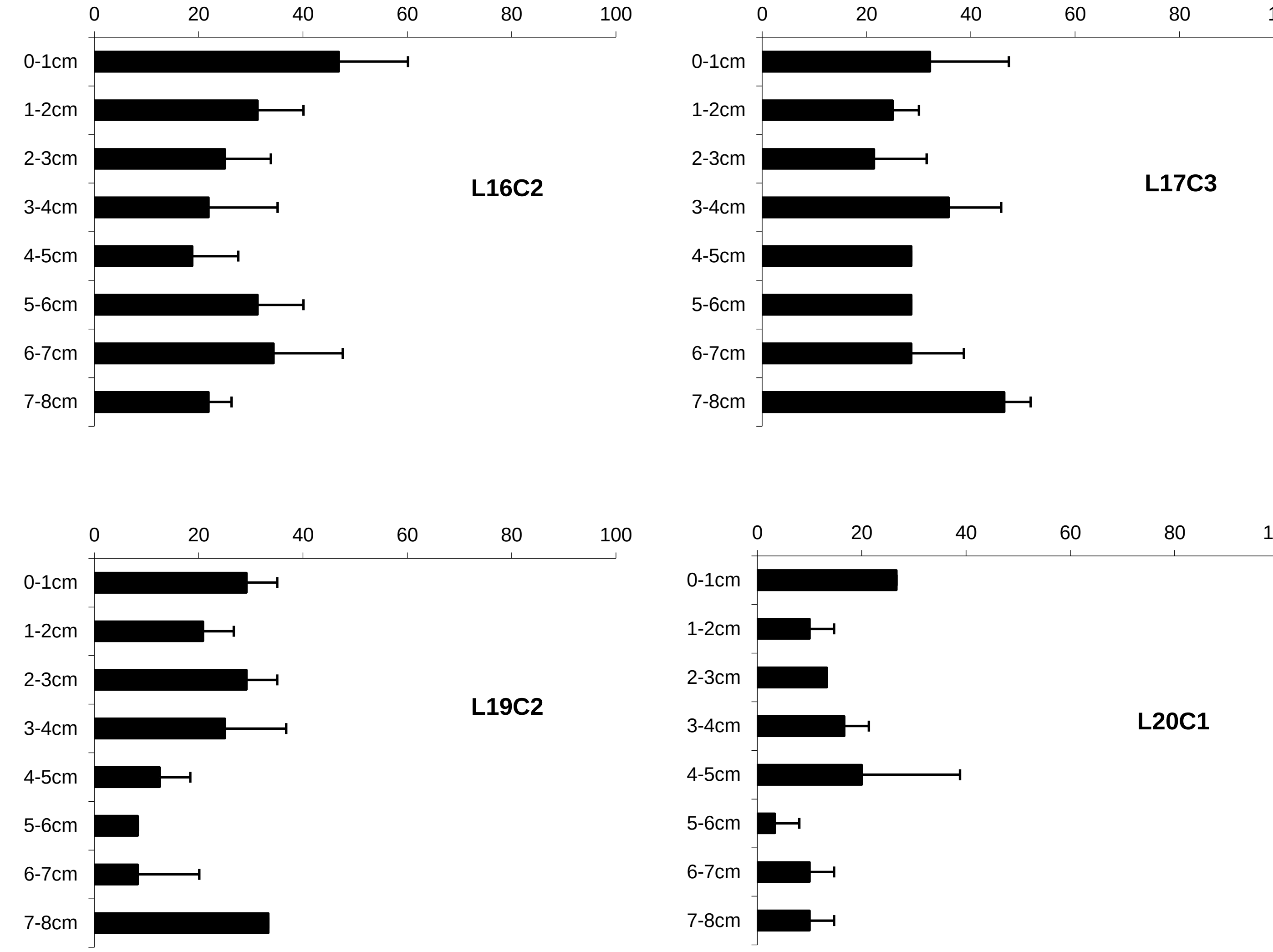
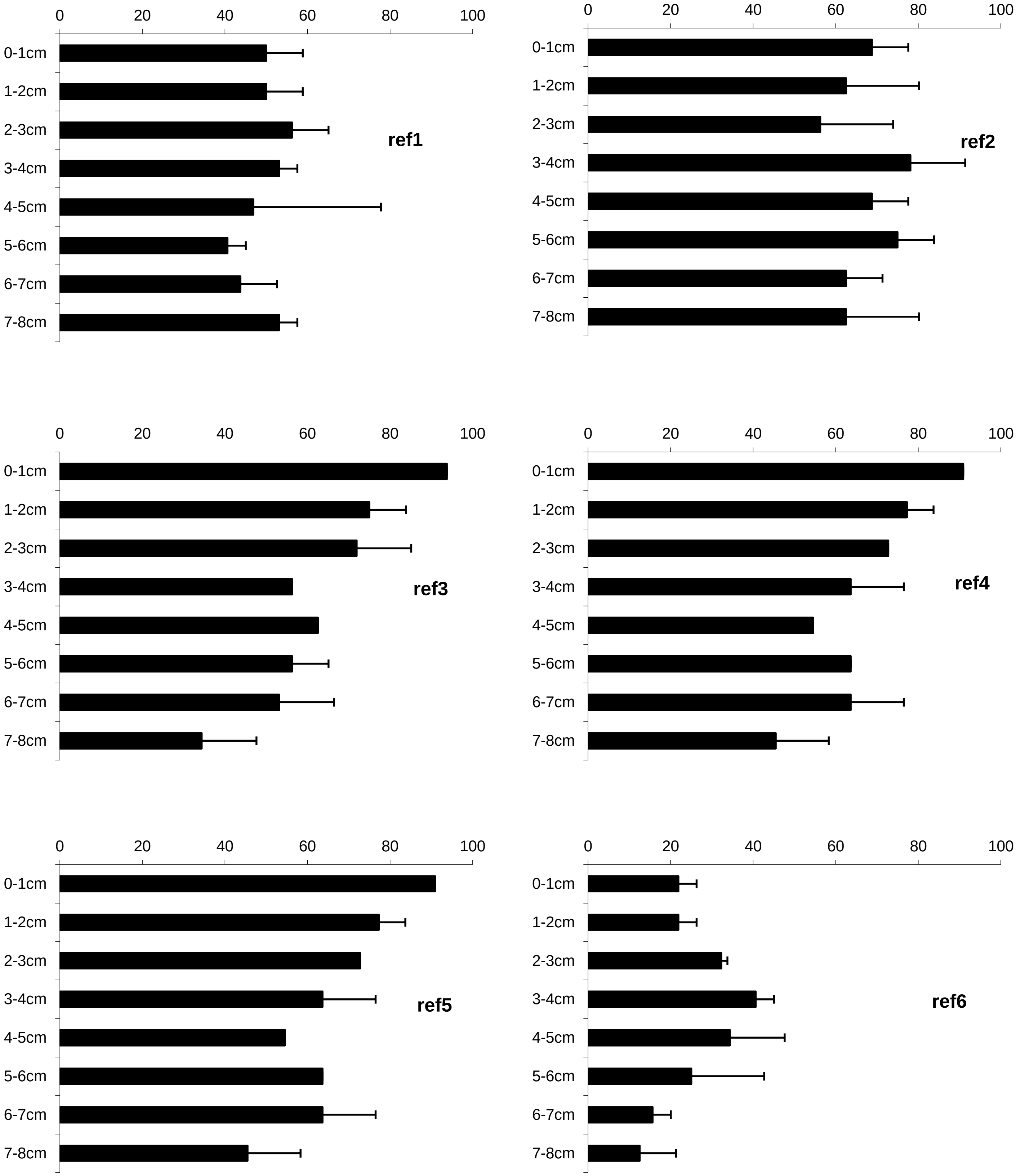


Figure NS8.28 – Average of percentage of totally or partially eaten holes (mean ± standard deviation) of a group of 16 bait lamina in the different depths, for the different sampling plots spread in the study site exposed for 90 days during the summer/autumn.



Phy2SUDOE project (SOE4/P5/E1021) is financed by the Interreg Sudoe Programme through the European Regional Development Fund (ERDF).

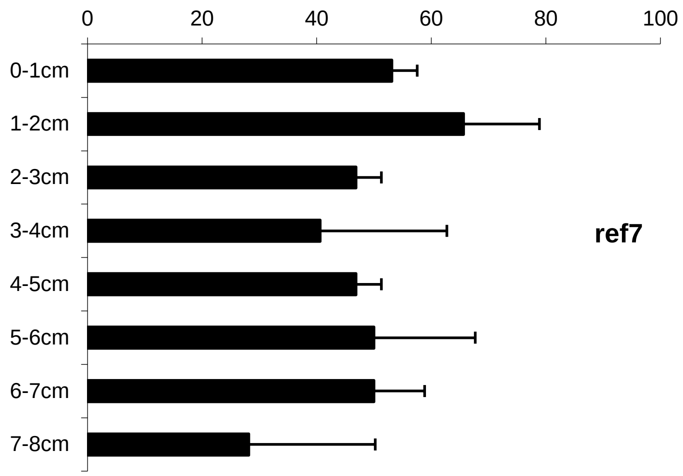
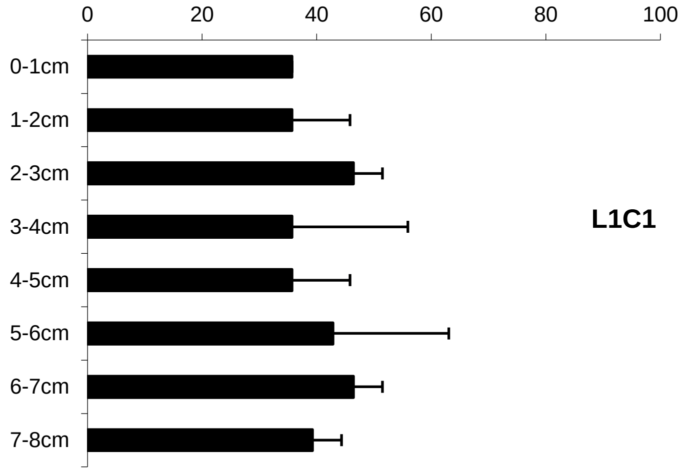


Figure NS8.29 – Average of percentage of totally or partially eaten holes (mean ± standard deviation) of a group of 16 bait lamina in the different depths, for the different sampling plots spread in the reference site exposed for 34 days.



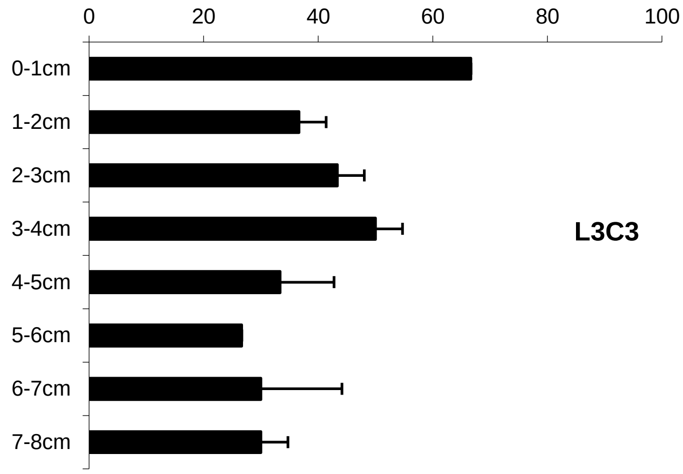


Figure NS8.30– Average of percentage of totally or partially eaten holes (mean ± standard deviation) of a group of 16 bait lamina in the different depths, for the different sampling plots spread in the study site exposed for 34 days.

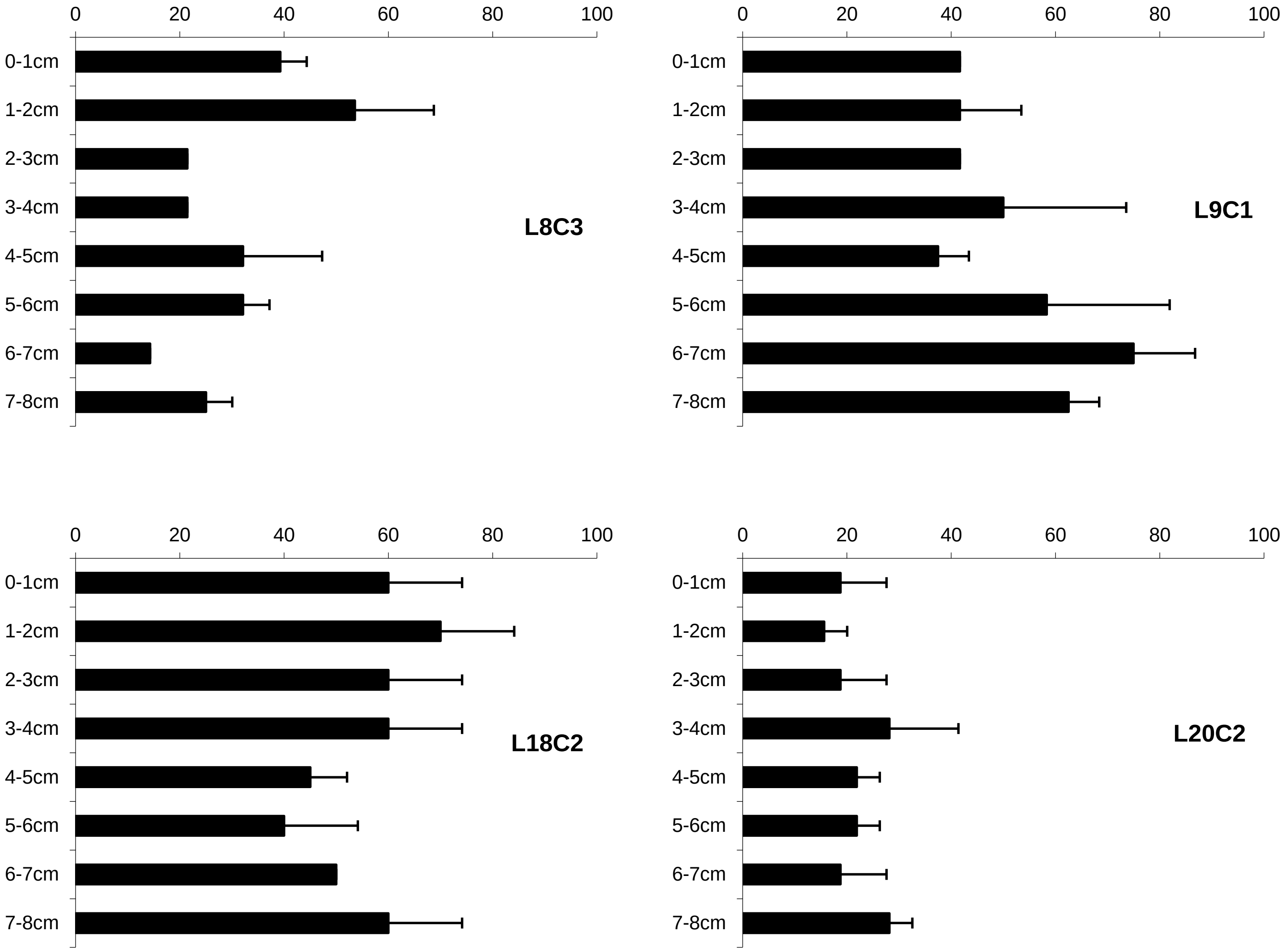


Figure NS8. 20 - photos of trap placement, collection, and identification of organisms

Appendices 5 – Number of juveniles (*eisenia andrei* - reproduction test)

Campaign 1 - test 1		Campaign 1 - test 2		Campaign 2 - test 1		Campaign 2- test 2	
Replicate	**number of juveniles**	**Replicate**	**number of juveniles**	**Replicate**	**number of juveniles**	**Replicate**	**number of juveniles**
CtR1	189	CtR1	139	CtR1	145	CtR1	105
CtR2	152	CtR2	157	CtR2	153	CtR2	85
CtR3	161	CtR3	113	CtR3	168	CtR3	78

Campaign 1 - test 1		Campaign 1 - test 2		Campaign 2 - test 1		Campaign 2- test 2	
Replicate	**number of juveniles**	**Replicate**	**number of juveniles**	**Replicate**	**number of juveniles**	**Replicate**	**number of juveniles**
CtR4	168	CtR4	184	CtR4	143	CtR4	114
SrefR1		SrefR1		SrefR1	177	SrefR1	
SrefR2		SrefR2		SrefR2	187	SrefR2	
SrefR3		SrefR3		SrefR3	170	SrefR3	
SrefR4		SrefR4		SrefR4	181	SrefR4	
L1R1	135	L11R1	147	L1R1	169	L11R1	142
L1R2	154	L11R2	151	L1R2	162	L11R2	208
L1R3	184	L11R3	145	L1R3	135	L11R3	125
L1R4	204	L11R4	149	L1R4	158	L11R4	152
L2R1	202	L12R1	151	L2R1	158	L12R1	0
L2R2	155	L12R2	156	L2R2	170	L12R2	0
L2R3	147	L12R3	151	L2R3	178	L12R3	106
L2R4	147	L12R4	97	L2R4	147	L12R4	4
L3R1	168	L13R1	148	L3R1	179	L13R1	104
L3R2	142	L13R2	198	L3R2	156	L13R2	113
L3R3	138	L13R3	153	L3R3	188	L13R3	179
L3R4	175	L13R4	136	L3R4	146	L13R4	157
L4R1	138	L14R1	122	L4R1	148	L14R1	108
L4R2	177	L14R2	125	L4R2	168	L14R2	168
L4R3	147	L14R3	142	L4R3	151	L14R3	105
L4R4	137	L14R4	126	L4R4	138	L14R4	123
L5R1	150	L15R1	152	L5R1	177	L15R1	136
L5R2	161	L15R2	146	L5R2	159	L15R2	123
L5R3	134	L15R3	159	L5R3	155	L15R3	142
L5R4	137	L15R4	126	L5R4	139	L15R4	97
L6R1	71	L16R1	57	L6R1	145	L16R1	109
L6R2	142	L16R2	92	L6R2	166	L16R2	154
L6R3	90	L16R3	97	L6R3	159	L16R3	123
L6R4	82	L16R4	92	L6R4	147	L16R4	152
L7R1	180	L17R1	129	L7R1	128	L17R1	125
L7R2	148	L17R2	112	L7R2	60	L17R2	134
L7R3	137	L17R3	124	L7R3	64	L17R3	89
L7R4	160	L17R4	107	L7R4	107	L17R4	126
L8R1	148	L18R1	113	L8R1	172	L18R1	98
L8R2	182	L18R2	98	L8R2	196	L18R2	170
L8R3	182	L18R3	96	L8R3	182	L18R3	120
L8R4	158	L18R4	125	L8R4	191	L18R4	100

Campaign 1 - test 1		Campaign 1 - test 2		Campaign 2 - test 1		Campaign 2- test 2	
Replicate	number of juveniles	Replicate	number of juveniles	Replicate	number of juveniles	Replicate	number of juveniles
L9R1	168	L19R1		L9R1	160	L19R1	120
L9R2	161	L19R2		L9R2	149	L19R2	118
L9R3	124	L19R3		L9R3	127	L19R3	136
L9R4	140	L19R4		L9R4	138	L19R4	99
L10R1	189	L20R1	63	L10R1	173	L20R1	132
L10R2	164	L20R2	76	L10R2	188	L20R2	127
L10R3	150	L20R3	79	L10R3	210	L20R3	135
L10R4	153	L20R4	54	L10R4	163	L20R4	123

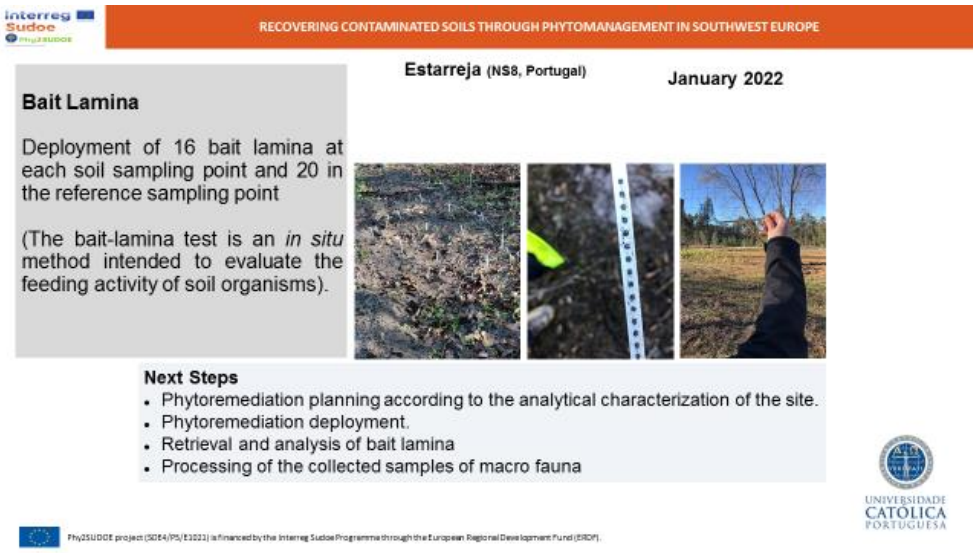
- **Phytomanagement options/ plant assembly**

The site is undergoing a major remediation project since 2021. On the southern boundaries of the large intervention area, a plot has been established for assessing phytomanagement options. The objective is to phytostabilize the metal(loid)s and to promote the degradation of the organic contaminants (PAHs, BTEX).

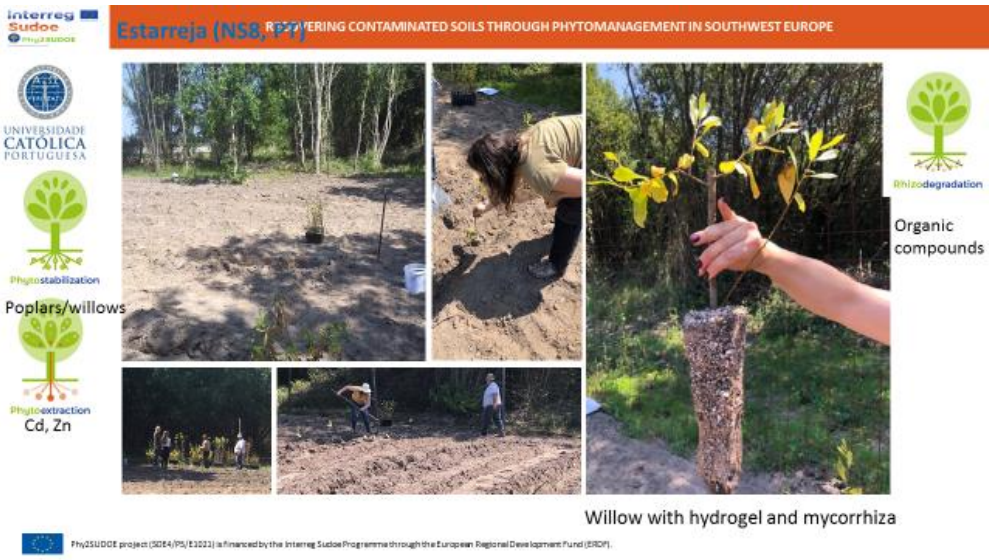
Cutting of poplars and willow (Cu/PAH-tolerant populations from INRAE) were implemented with mycorrhizae and either intercropped with vetiver (poplars) or cultivated on soil amended with hydrogel (willows).

- **Success / limits**

Sampling of soil and soil macro-fauna using Pitfall traps were carried out. The bait-lamina test was used as an in situ method intended to evaluate the feeding activity of soil organisms in the plot topsoils. Poplars and willows, with and without mycorrhizae and hydrogel, and vetiver (intercropped with poplar) were implemented in an irrigated field trial.



Deployment of bait-lamina and field trial at the Estarreja site (© UCP)



Transplantation of mycorrhizal willows at the Estarreja site © UCP