RECOVERING CONTAMINATED SOILS THROUGH PHYTOMANAGEMENT IN SOUTHWEST EUROPE

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

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

Product 2.1/E.2.1.1 Phy2Sudoe network enlargement - Characterization of new sites

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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 varied 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 there according to the PhytoSUDOE and Phy2SUDOE methodologies, 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 neighbouring 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, in collaboration with CD64, Bordes town and Suez lyre)
- PT: Estarreja - industrial area: mixed pollution (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		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	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 – Gernika (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

● 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

● Methodologies for characterizing physico-chemical and biological activities are listed in the following tables 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				
	CEC	**Cobaltihexamine method**	X	X	X	X				
		1M NH4Cl (Ca, Mg, Na, K), 1M KCl (H, Al), AAS/ICP-OES								
	Extractable P (Olsen's NaHCO3method)		X (Olsen)	X (joret - Hébert	X (Olsen)	X (Olsen)				
	Phosphorus speciation/fractionation									
	Total C and N (Combustion, LECO analyzer)		X	X	X	X				
	SOM fractionation									
	Carbonates (Gravimetry, Schleiber method)		X	x	X	X				
	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,..)	**NH4Cl**								
		NH4NO3	x	x	x	x				
		H2O								
		EDTA								
	Total TE (H2O2/3:1 HCl:HNO3, microwave)		X (HF)	X (Microwave)	X (HF)	X (HF)			X (VIE-B)	
	Soil metal fractionation (modified BCR protocol)									
	Total PAH (Hexane extraction, GC-MS determination)		x			X			X (VIE-B)	
	other organic contaminants		PCB, VOC (TCE), BTEX			PCB, BTEX			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		
	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**	
	TE concentration		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				
	TEs bioconcentration and translocation factors		X		X	X				
	Soil health cards								X	

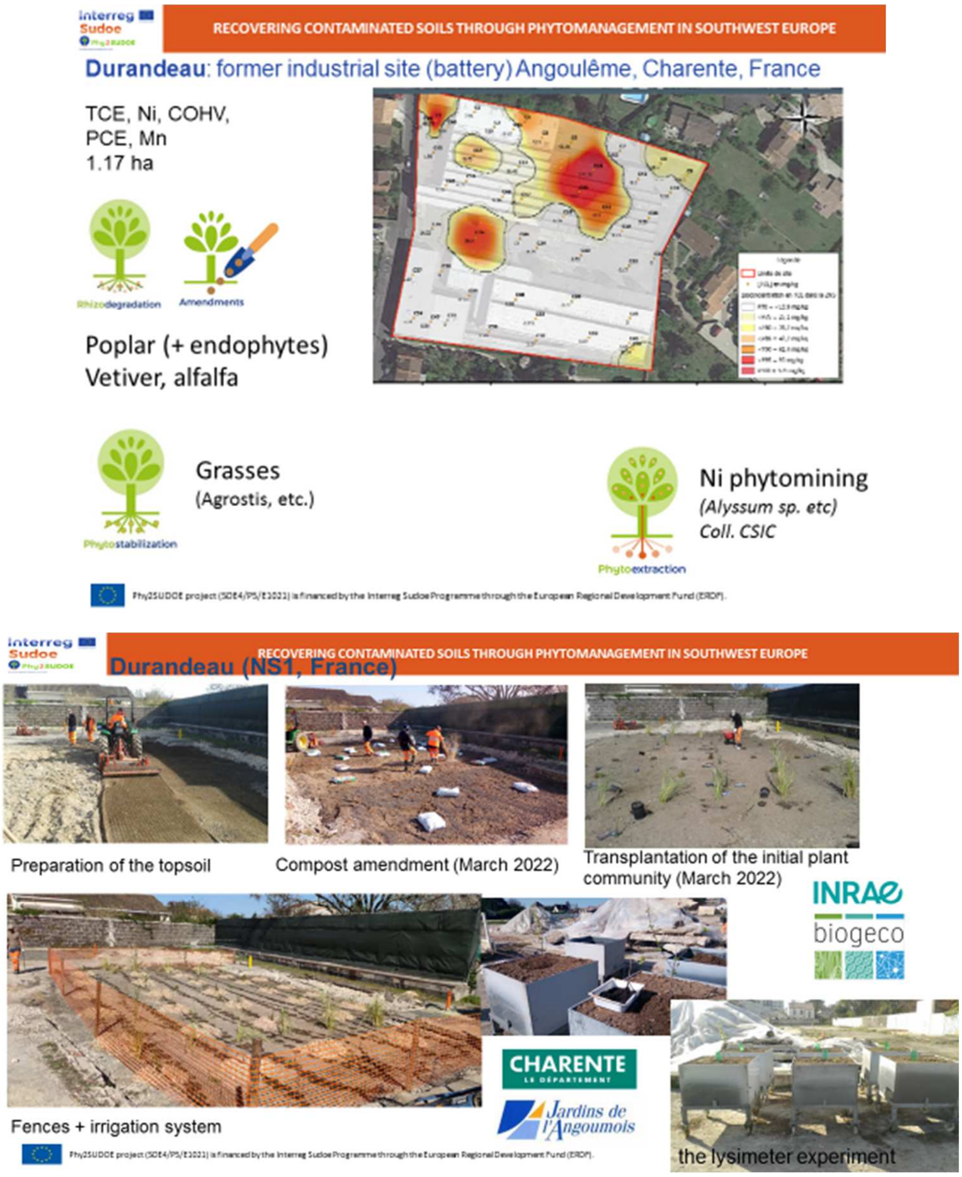
* leaf area index
maximum shoot length
DW yield of plant parts

** total photosyntethic area
leaf area index
maximum shoot length
DW yield of plant parts

*** photosyntethic efficiency
chlorohyll
carotenoids
tocopherol

NS1 Durandeau site:

● **Implementation**: organic and inorganic contaminants were firstly assessed by La Charente (and HPC Environtec) 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:

Durandeau (NS1, France)

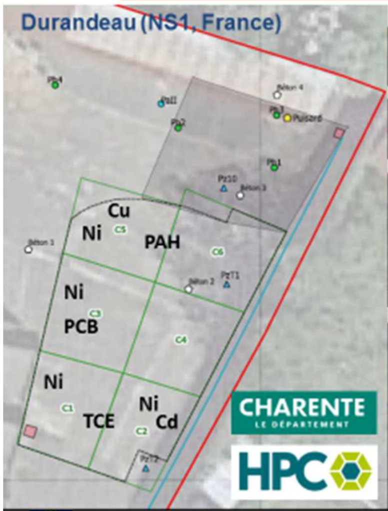
Feb – May 2022

- 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

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



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	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Ethylbenzène	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
o-Xylène	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
m,p-Xylène	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Xylènes totaux	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Somme des 4 BTEX	mg/kg MS	<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)	mg/kg MS	**54,6**	**93,1**	**52,4**	**60,1**	**68,8**	**25,3**	**41,4**
Chrome (Cr)	mg/kg MS	17,6	17	17,9	28,1	19	16,7	18,9
Cuivre (Cu)	mg/kg MS	**63**	58,7	49,9	58,3	**129**	**112**	54,3
Mercure (Hg)	mg/kg MS	1,11	**3,05**	2,06	1,23	**3,5**	**2,54**	1,0
Nickel (Ni)	mg/kg MS	1 780	1 900	2 440	973	4 070	<1,00	2 580
Plomb (Pb)	mg/kg MS	**135**	**100**	**108**	84,9	**194**	**159**	90,9
Zinc (Zn)	mg/kg MS	124	**361**	159	177	**324**	**308**	145

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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	mg/kg MS	0,01	0,01	2,05	0,02	0,89	0,89	0,2
PCB 101	mg/kg MS	0,05	0,05	6,45	0,07	2,16	3,91	0,55
PCB 118	mg/kg MS	0,05	0,03	3,04	0,06	1,29	1,82	0,03
PCB 138	mg/kg MS	0,2	0,13	33,3	0,16	4,14	8,03	1,12
PCB 153	mg/kg MS	0,19	0,14	22,2	0,14	4,91	8,4	1,41
PCB 180	mg/kg MS	0,12	0,07	14	0,06	2,59	3,57	0,59
Somme des 7 PCB	mg/kg MS	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	mg/kg MS	<0,05	<0,05	<0,05	<0,05	<0,25	0,51	<0,05
Acénaphtène	mg/kg MS	<0,05	<0,05	<0,05	<0,05	0,5	2,3	0,28
Fluorène	mg/kg MS	<0,05	<0,05	<0,05	0,06	0,28	1,2	0,13
Phénanthrène	mg/kg MS	<0,05	0,13	0,1	0,45	3,4	5	0,92
Anthracène	mg/kg MS	<0,05	<0,05	<0,05	0,1	1	2,5	0,45
Fluoranthène	mg/kg MS	0,09	0,18	0,27	0,67	6,5	15	2,3
Pyrène	mg/kg MS	0,11	0,17	0,25	0,47	5,4	14	2
Benzo(a)anthracène	mg/kg MS	0,10	0,09	0,16	0,31	3,3	9,6	0,96
Chrysène	mg/kg MS	0,13	0,12	0,19	0,36	3,9	9,4	0,94
Benzo(b)fluoranthène	mg/kg MS	0,31	0,26	0,39	0,46	10	20	1,7
Benzo(k)fluoranthène	mg/kg MS	0,09	0,09	0,13	0,18	3,3	6,4	0,63
Benzo(a)pyrène	mg/kg MS	0,17	0,18	0,24	0,25	6,6	15	1,2
Indéno(1,2,3-c,d)pyrène	mg/kg MS	0,2	0,18	0,25	0,29	7,2	15	1,3
Dibenzo(a,h)anthracène	mg/kg MS	<0,05	<0,05	0,06	0,07	1,6	3	0,21
Benzo(g,h,i)pérylène	mg/kg MS	0,18	0,24	0,23	0,32	6,6	14	1,1
Somme des 16 HAP	mg/kg MS	1,38	1,64	2,35	3,98	60,12	133,53	14,56

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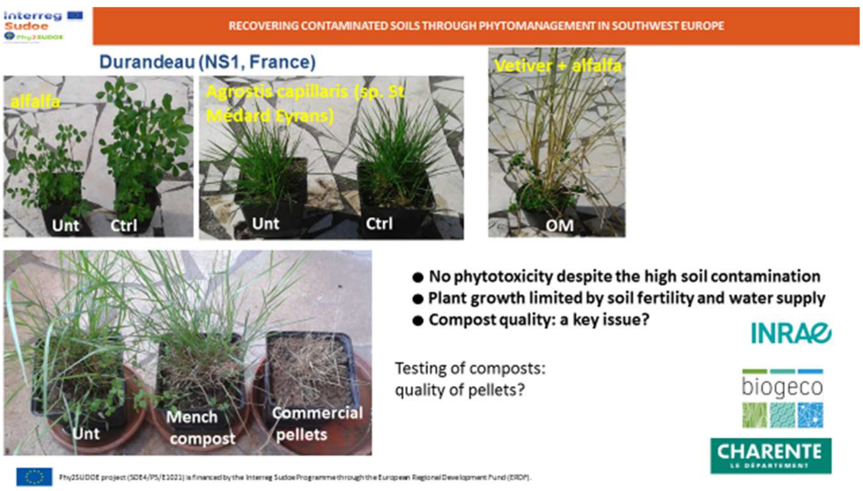
Composés Organo Halogénés volatils [COHV]		C1	C2	C3	C4	C5	C6	C7
		0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5	0 - 0,5
cis 1,2-Dichloroéthylène	mg/kg MS	<0,10	0,28	<0,10	<0,10	<0,10	0,17	<0,10
Trans-1,2-dichloroethylène		<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
Trichloroéthylène		3,36	8,62	0,62	0,38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

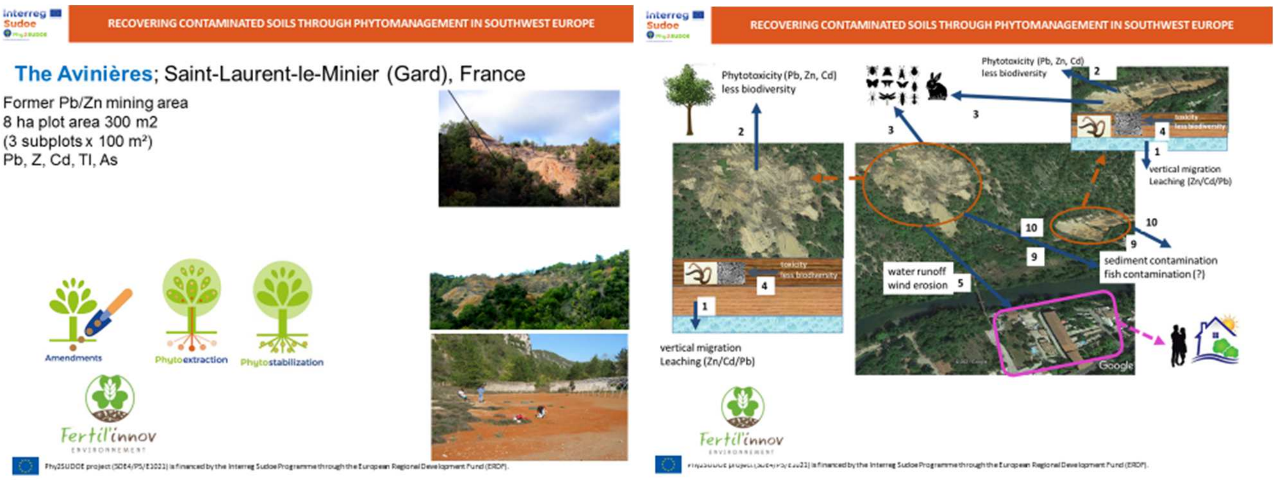
● **implementation**:

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.

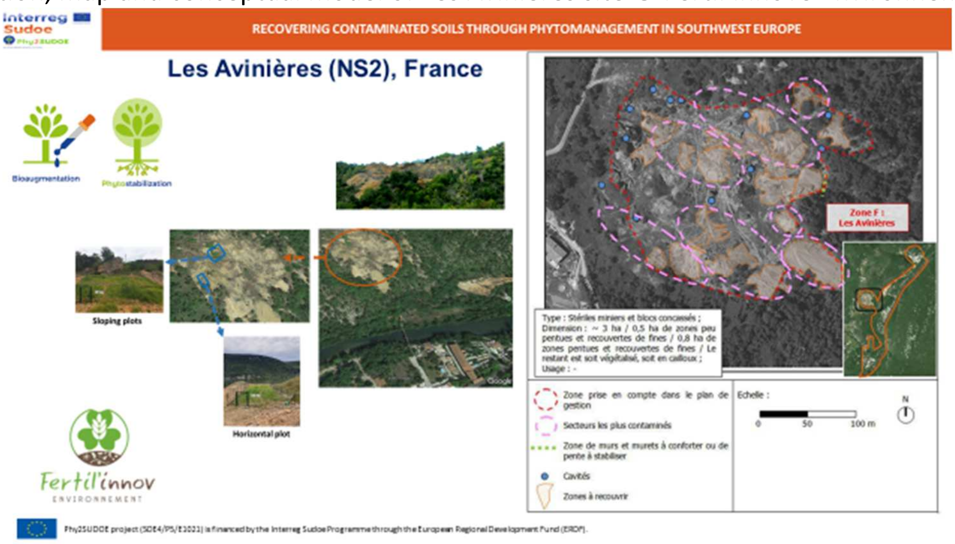
The risk of contamination of humans by ingestion or direct contact is very high. Wind erosion brings metal-laden dust into houses. During the rainy season, when small puddles are created, contaminants can pass from water to wild animals (deer, foxes, small mammals, amphibians, reptiles, etc.) by ingestion 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'innove Environnement



Location of the field plots of Les Avinières site © Fertil'innove 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.

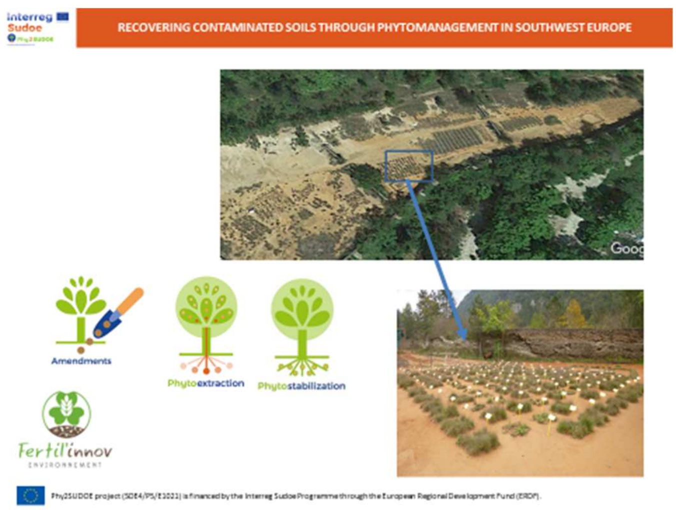
The metal(loid) contents in the former mining area are in excess. For the mining waste rock sector, these total soil contents ranged from 7 to 3300 mg As/ kg DM, from 22 to 1200 mg Cd/ kg DM, from 0 to 0.22 mg Hg / kg DM, from 3,800 to 50,000 mg Pb/ kg DM, 12 to 83 mg Tl / kg DM, and 41,000 to 54,000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

● phytomanagement options /plant assembly

Several plant species have adapted to the soil and climatic conditions of the Avinières site and, despite the constraint of high levels of heavy metals, 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.

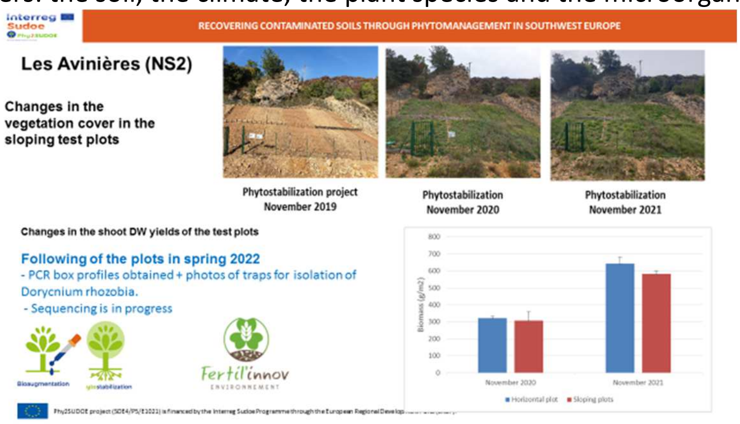
In situ tests were set up at the end of 2019 before proceeding with the rehabilitation of the old mine to confirm the effectiveness of the supply of organic matter and optimize the association of the various plant and microbial species. The pilot tests made it possible to define the phytostabilization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

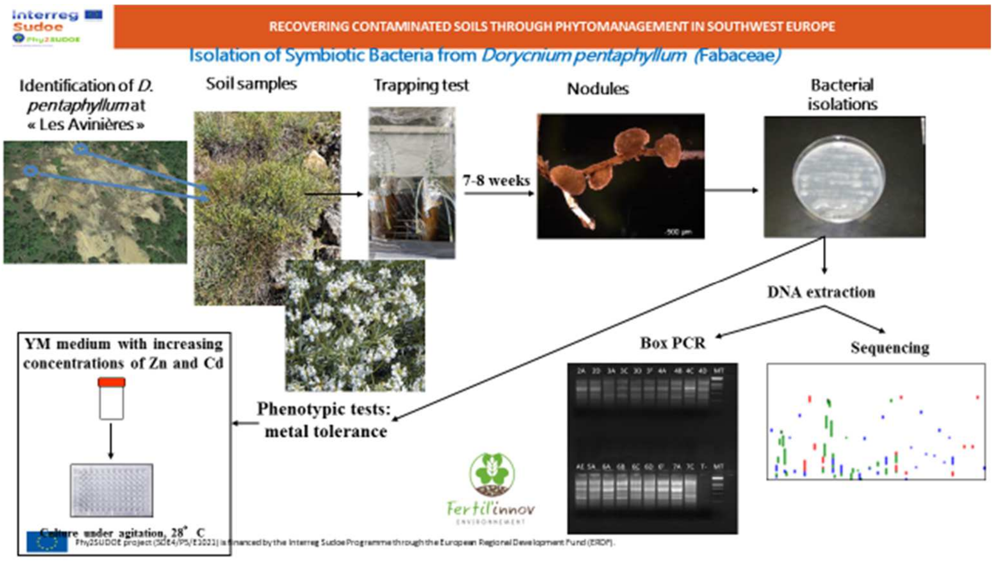


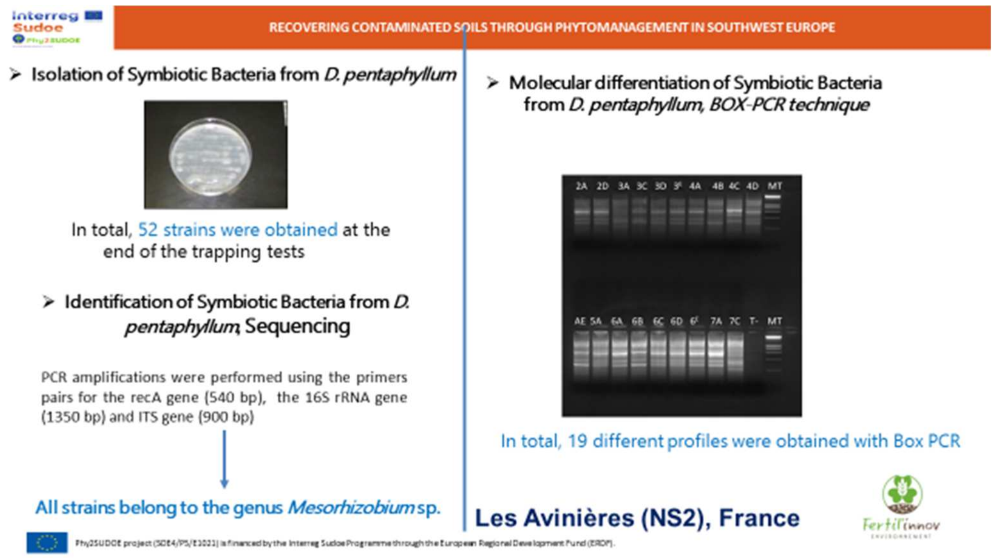
Focus on field plots at Les Avinières site © Fertil'innove Environnement

● **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.

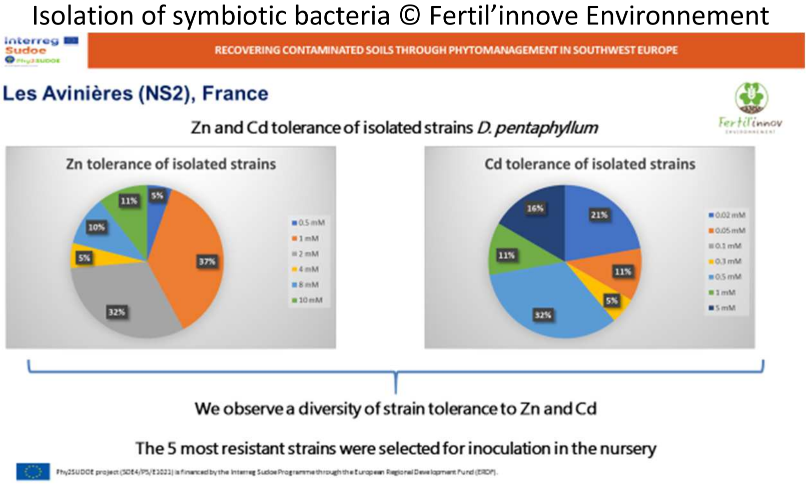
The Avinières site was revegetated in 2022 and the monitoring of the vegetation cover is continuing. The pilot tests demonstrated that it is possible to achieve the site phytostabilization while accounting for several parameters: the soil, the climate, the plant species and the microorganisms.

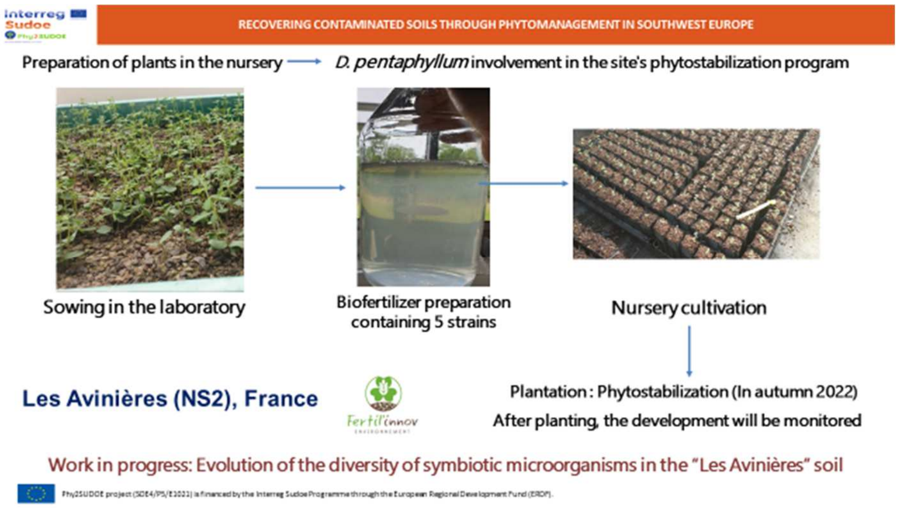






Isolation of symbiotic bacteria © Fertil'innove Environnement

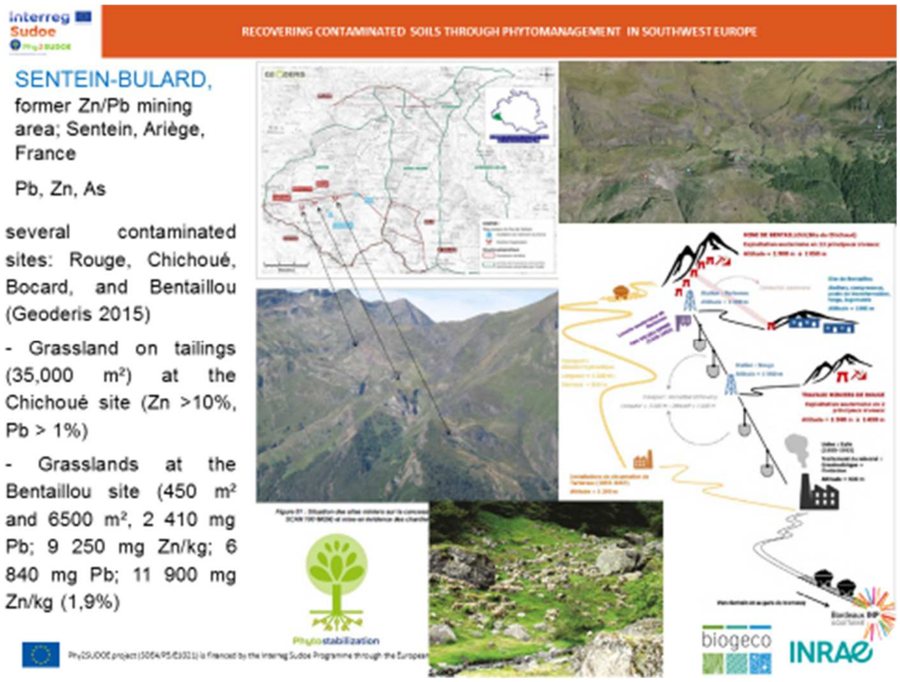




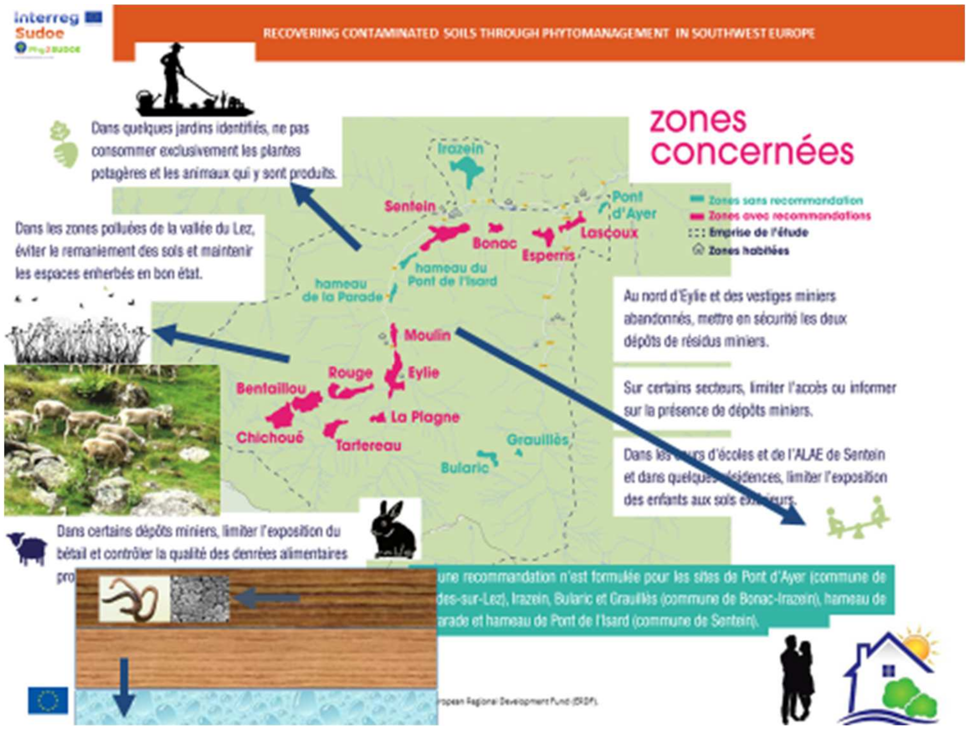
Dorycnium pentaphyllum and rhizobia nodules (© S. Soussou, Fertil'innove Environnement)

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.

Interreg Sudoe Phy2SUDOE

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

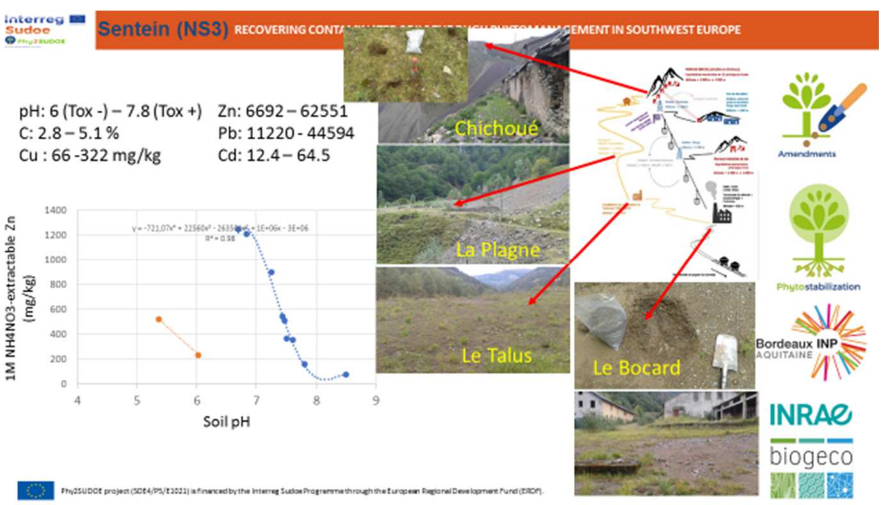
Physico-chemical properties of the Sentein sols © INRAE in coll. Bordeaux INP

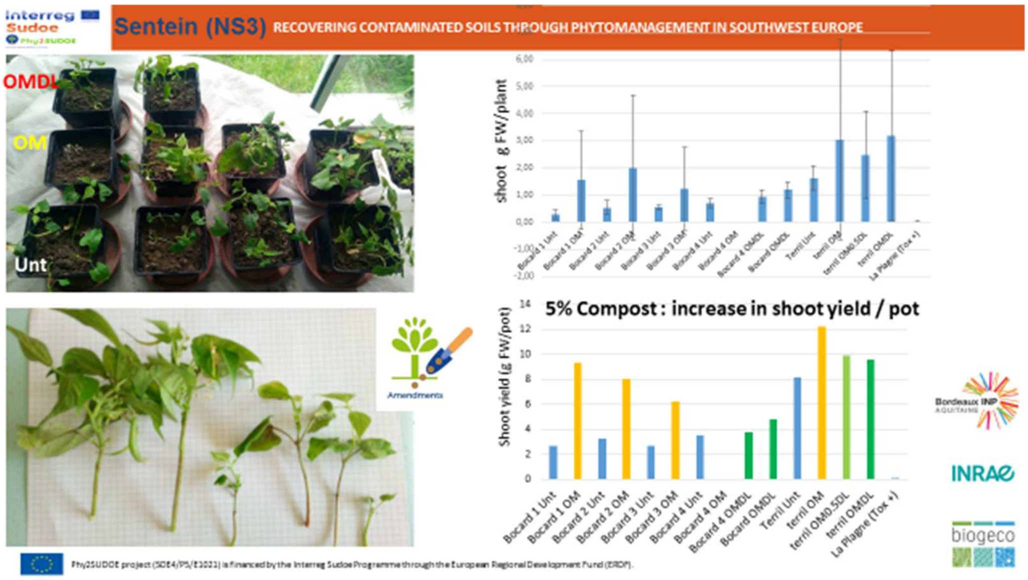
● **Phytomanagement option /plant assembly**

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 vermirediation 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'Innove 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:

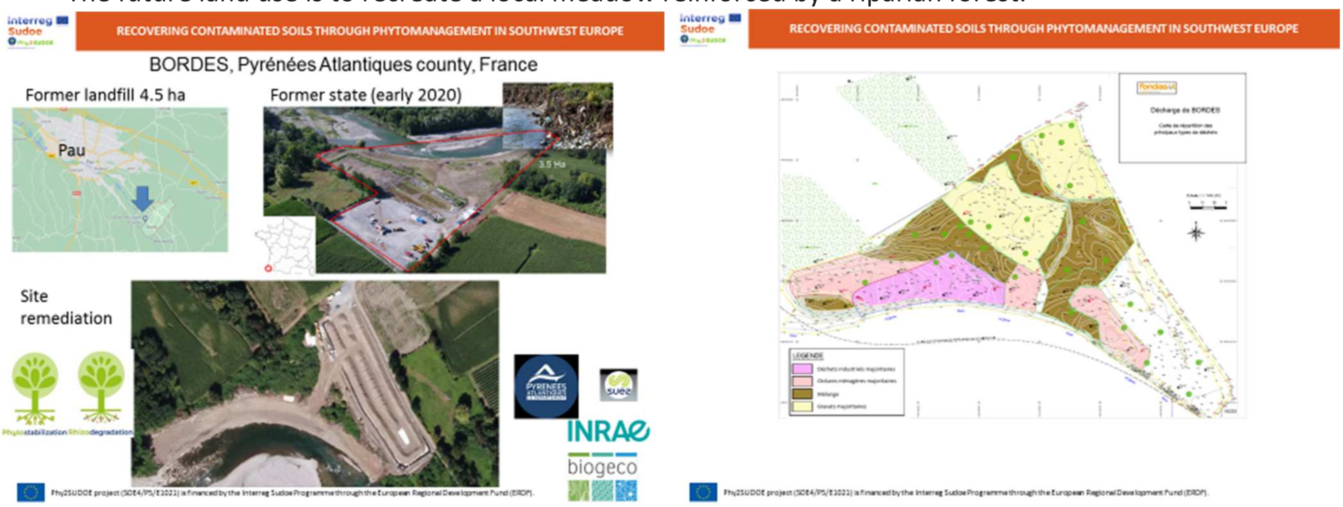
This site is a former landfill supervised by the Bordes town, the community of communes of the Pays de Nay, and the Pyrénées Atlantiques County.

● **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.

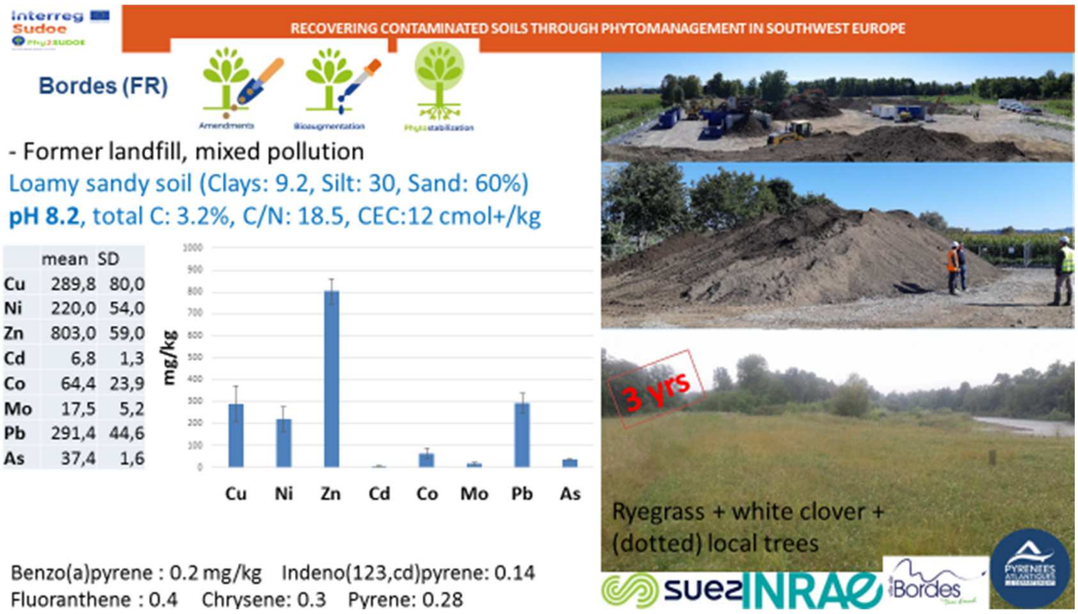
The future land use is to recreate a local meadow reinforced by a riparian forest.



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.

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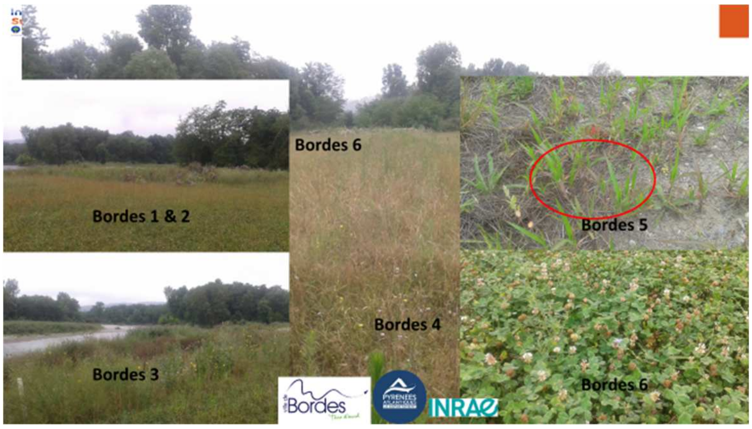
Local (native) grassy plant species (from northern Bearn meadows) and those used for green manure (rye, white clover and ryegrass) and local tree species were implemented;
The seed bank was increased by hay transfer (the succession should result in a Riparian forest).

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: *Fraxnus 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



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 an 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;

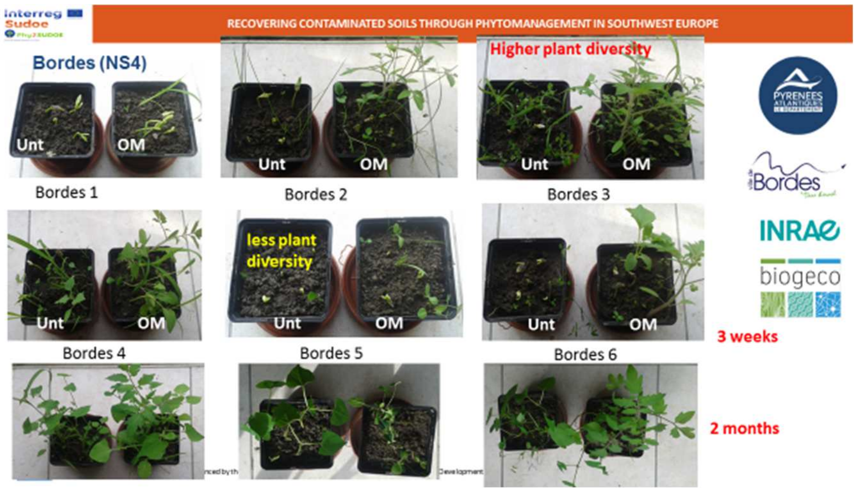
- The pot experiments carried out in 2022 with topsoils collected at this site has evidenced no remaining visible phytotoxicity on the shoots (on a sensitive plant species such as dwarf beans)

- 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

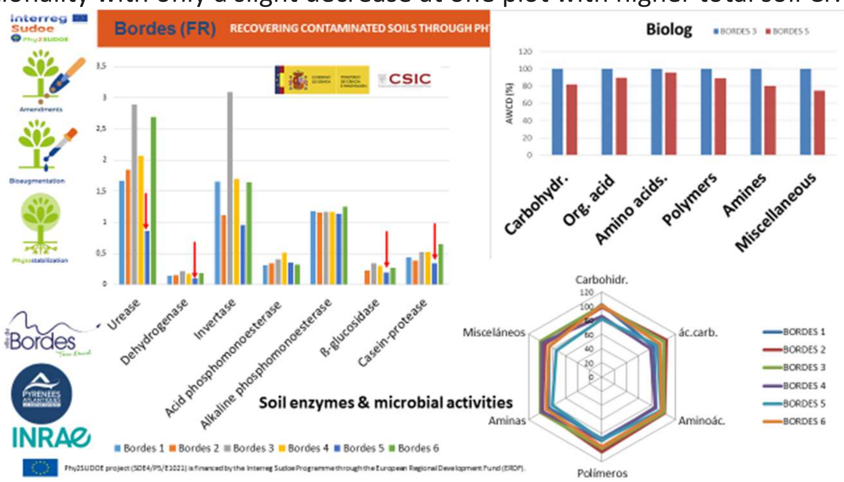
Butterfly on white clover on site and study of seed banks and soil phytotoxicity for the Bordes site © Mench/INRAE



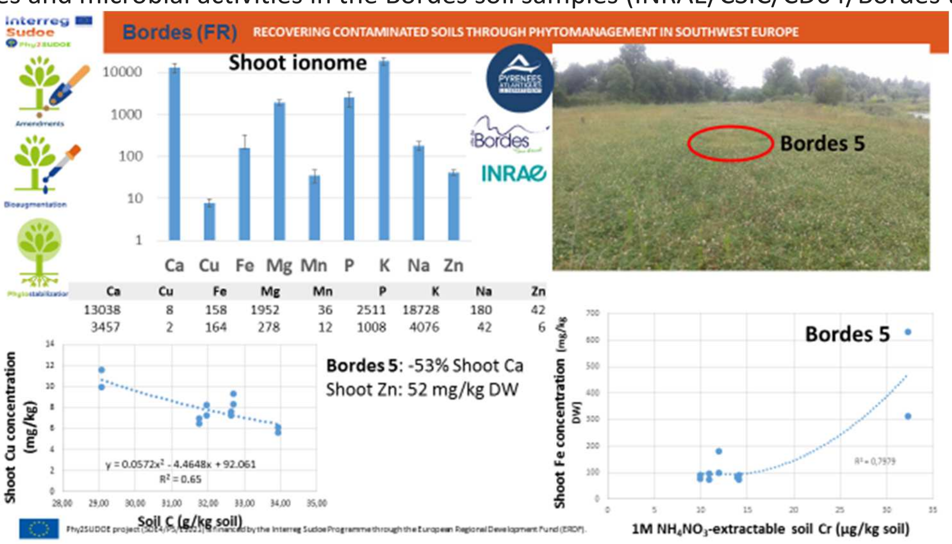
Plant testing of the Bordes soils with and without compost addition © Mench/INRAE

● 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)

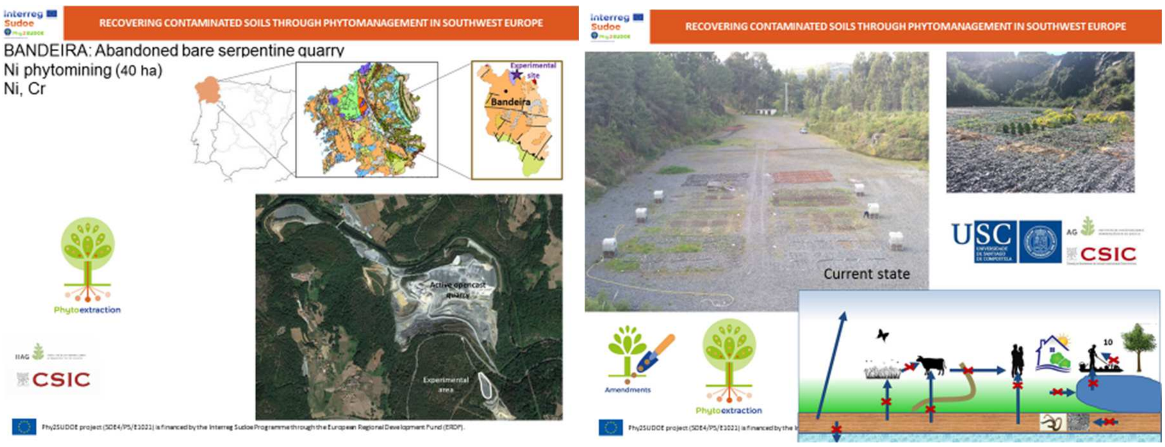


Shoot ionome of the vegetation cover at the Bordes sites © Mench INRAE/CD64

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 NH4NO3-extractable soil Cr and some purple symptoms on the leaf sheath of *Holcus lanatus*.

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 serpentinised 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.

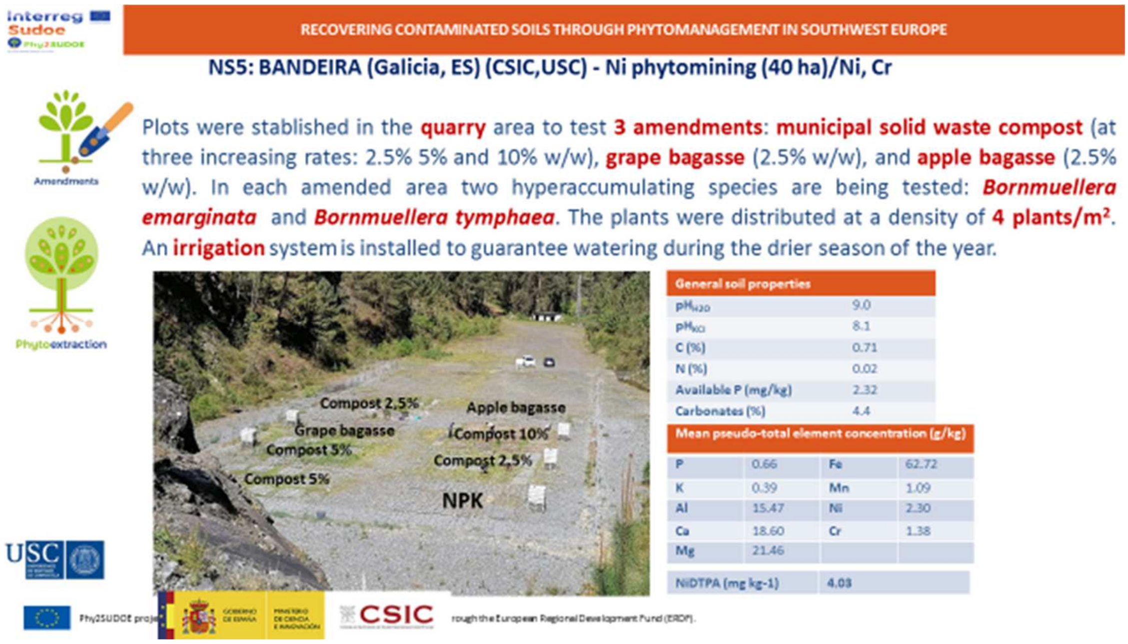


Location, field trial and conceptual model for the Bandeira site © CSIC-USC

● **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.



Physico-chemical soil properties at the Bandeira site © CSIC-USC

● **Phytomanagement options /plant assembly**

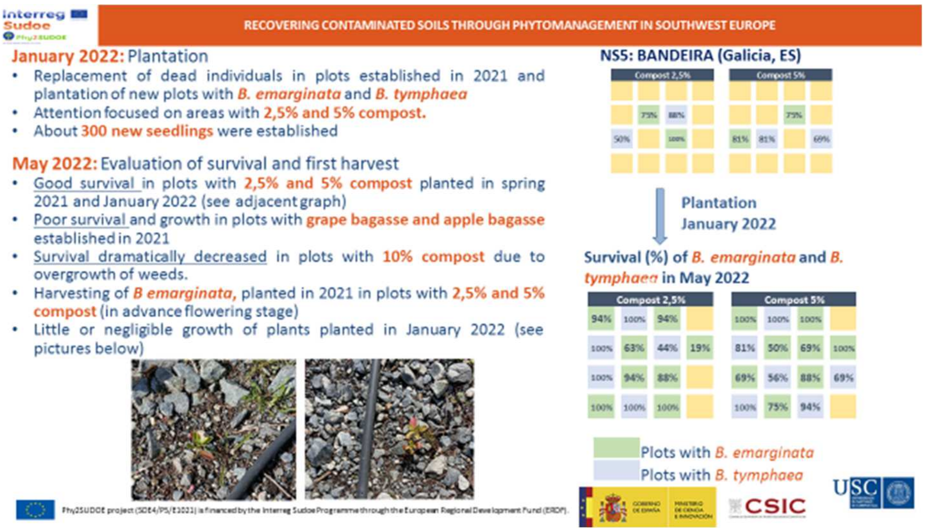
The experimental plots are located in one of the spoil heap of this quarry.

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.

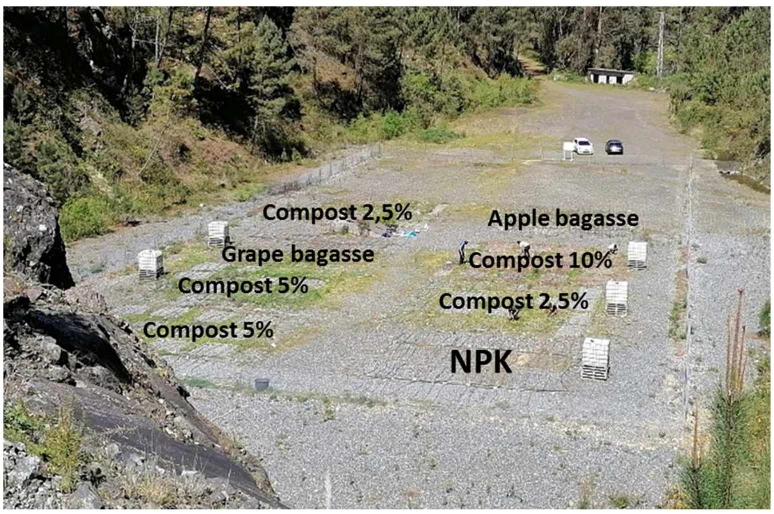
Plants established in spring 2021 were harvested in spring 2022. The biomass of *B. emarginata* generally reached 300 kg ha^{-1}. The Ni yield peaked with 2.5% compost amendment (about 1kg Ni ha^{-1}) as compared to soils receiving 5% compost (up to 0.4 kg Ni ha^{-1}).

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.

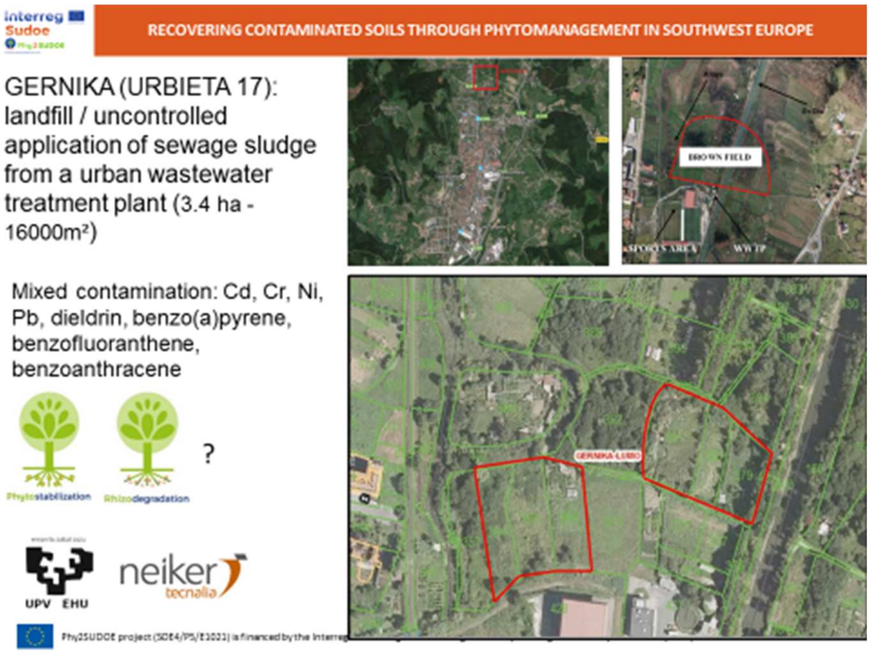
Limits are water supply and distribution along the year in line with climate change (heatwaves, drought) and the low water holding capacity of this soil. Implementation of the irrigation system was relevant.



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

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. The landfill 17, which has an inventory area of 3.38 Ha and 16,000 m2, 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. The 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).

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

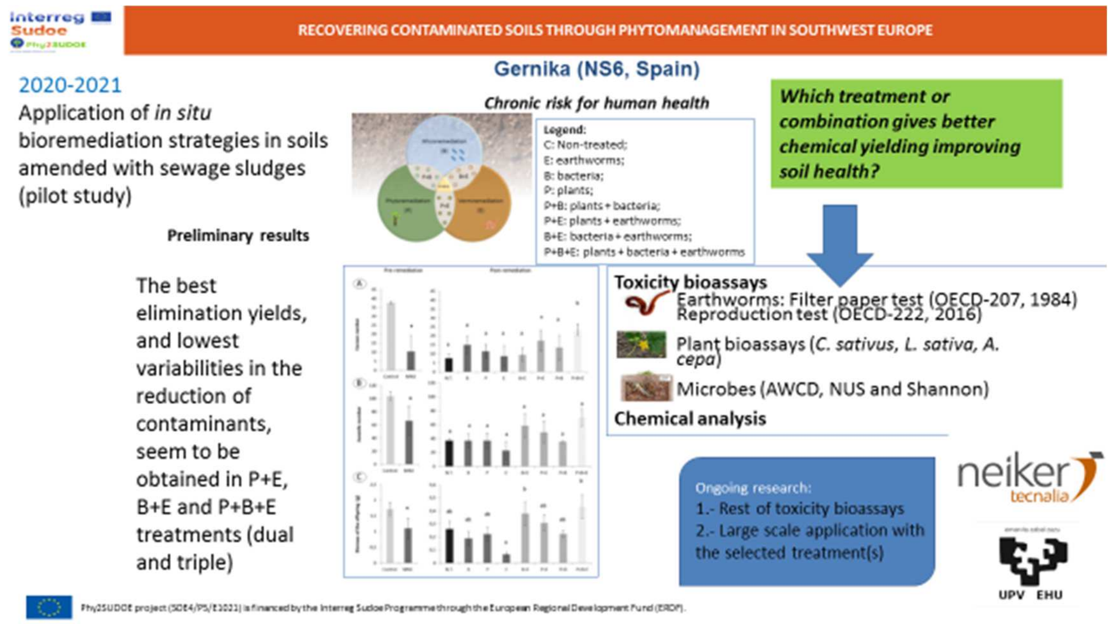


● **soil properties**: the soil displayed a mixed contamination with Cd, Cr, Ni, Pb, dieldrin, benzo(a)pyrene, benzofluoranthene and benzo anthracene in exces.

● **phytomanagement options :plant 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). 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.

● **success / limits**

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)

The most efficient phytomanagement option combines crop (e.g. alfalfa), bacterial consortium, and worms. 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.

NS7 Zumanakotxa:

● **Implementation**: 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. 7.1. Site cleaning setting aside residues (asphalt) and inert material (@Vilela, CEA)

Pict.7.2. Site preparation for poplar/willow stands (@Vilela, CEA)

● **Soil properties**

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 legislation (VIE-B levels). The compounds detected overpassing reference levels were aliphatic petrol hydrocarbons (TPH), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) and aldrin.

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.

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

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

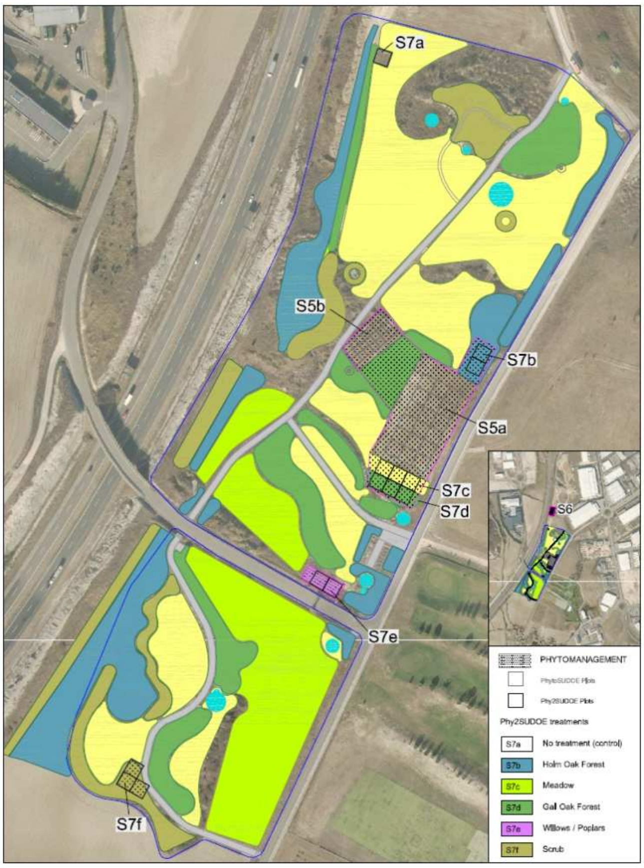
NS7e: poplar/willow/alder stands

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

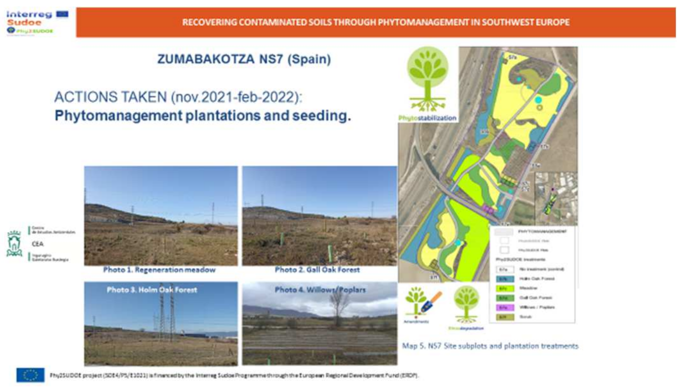


Fig. 7.3 Map and phytomanagement set-up (@Vilela, CEA)

● **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)

Plant analysis (EHU) is ongoing

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)

LINKS
TO CEA DOCUMENTS
Initial pollution analysis (2015 investigation)
Pollution analysis results before planting (xx/11/2020)
Pollution analysis results after planting (17/06/2022)
Pollution summary
Planting vegetation list (plant species)

NS - Estarreja, Portugal:

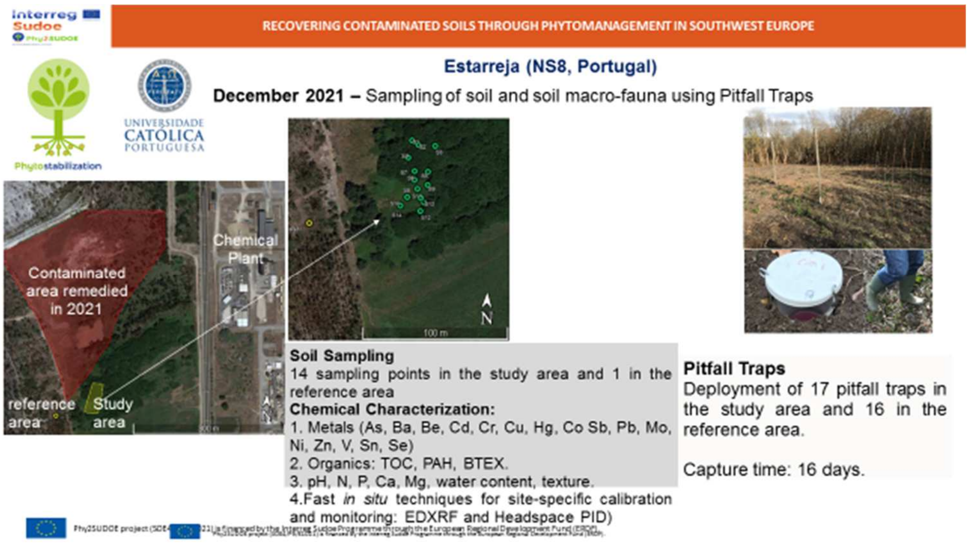
● Implementation

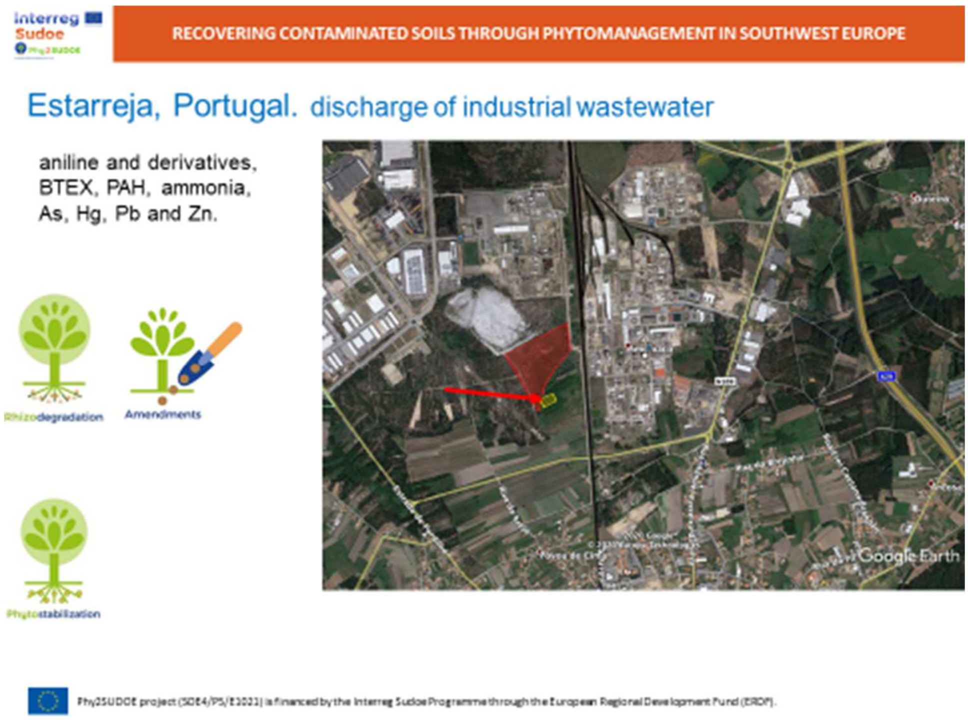
This site in the vicinity of a large chemical industrial complex is an area with high permeability and an average depth to groundwater level of less than 1 meter, with seasonal flooding due to the aquifer rise. This led to build a network of drainage ditches, which allowed the agricultural land use. 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.

● 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.

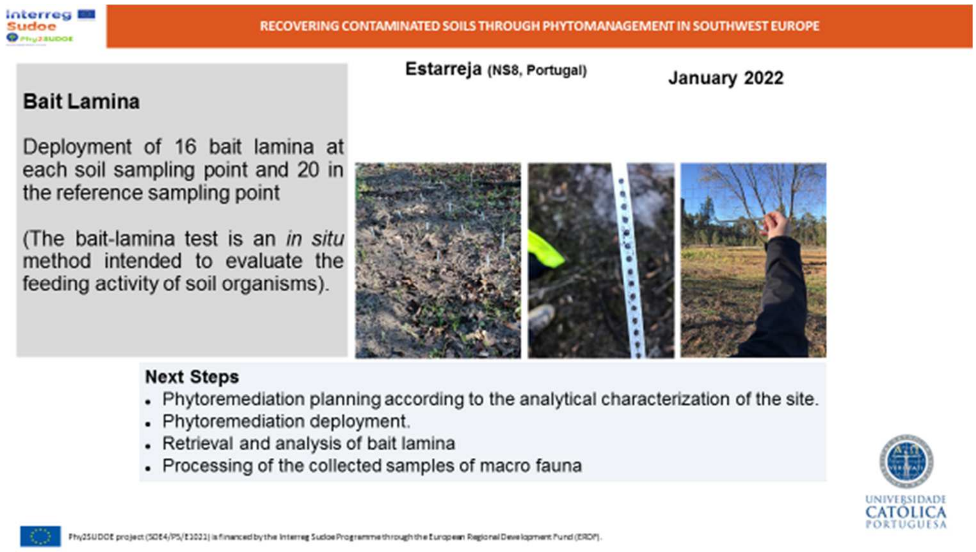
● **Phytomanagement options/ plant assembly**

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