

The use of organic and inorganic amendments in the phytoremediation of contaminated soils and their effect on biodiversity

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School of Soil Biodiversity and Bioindication

*Biodiversity and bioindicators in monitoring
and management of contaminated soils*



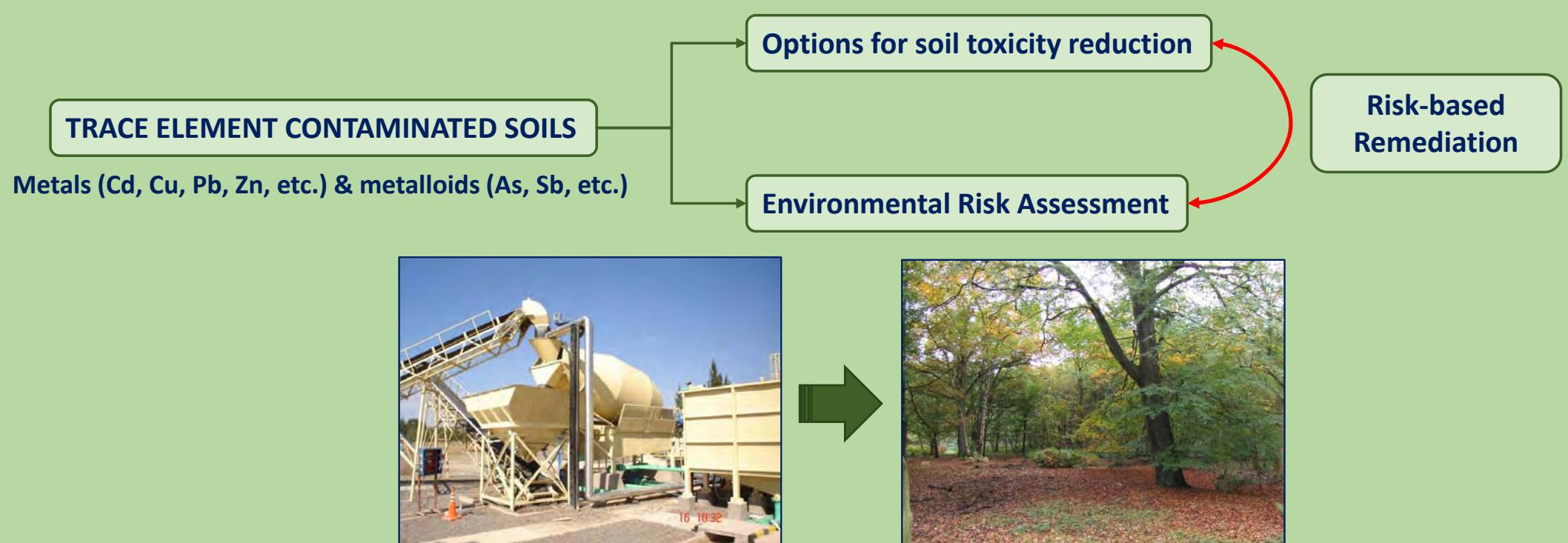
GOBIERNO
DE ESPAÑA

MINISTERIO
DE CIENCIA, INNOVACIÓN
Y UNIVERSIDADES

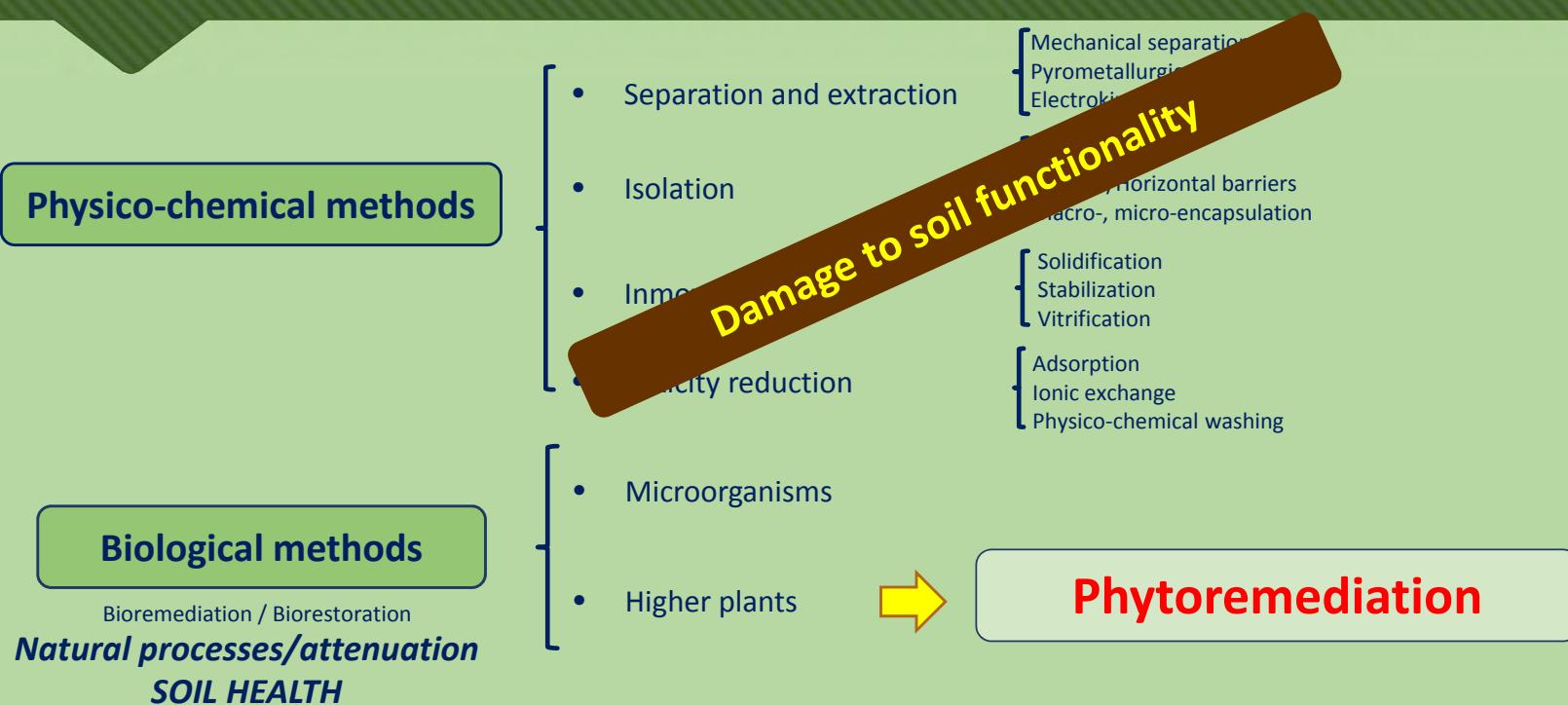


CEBAS
CENTRO DE EDAFOLOGÍA Y
BIOLOGÍA APLICADA DEL SEGURA

Trace elements - Contamination - Phytoremediation

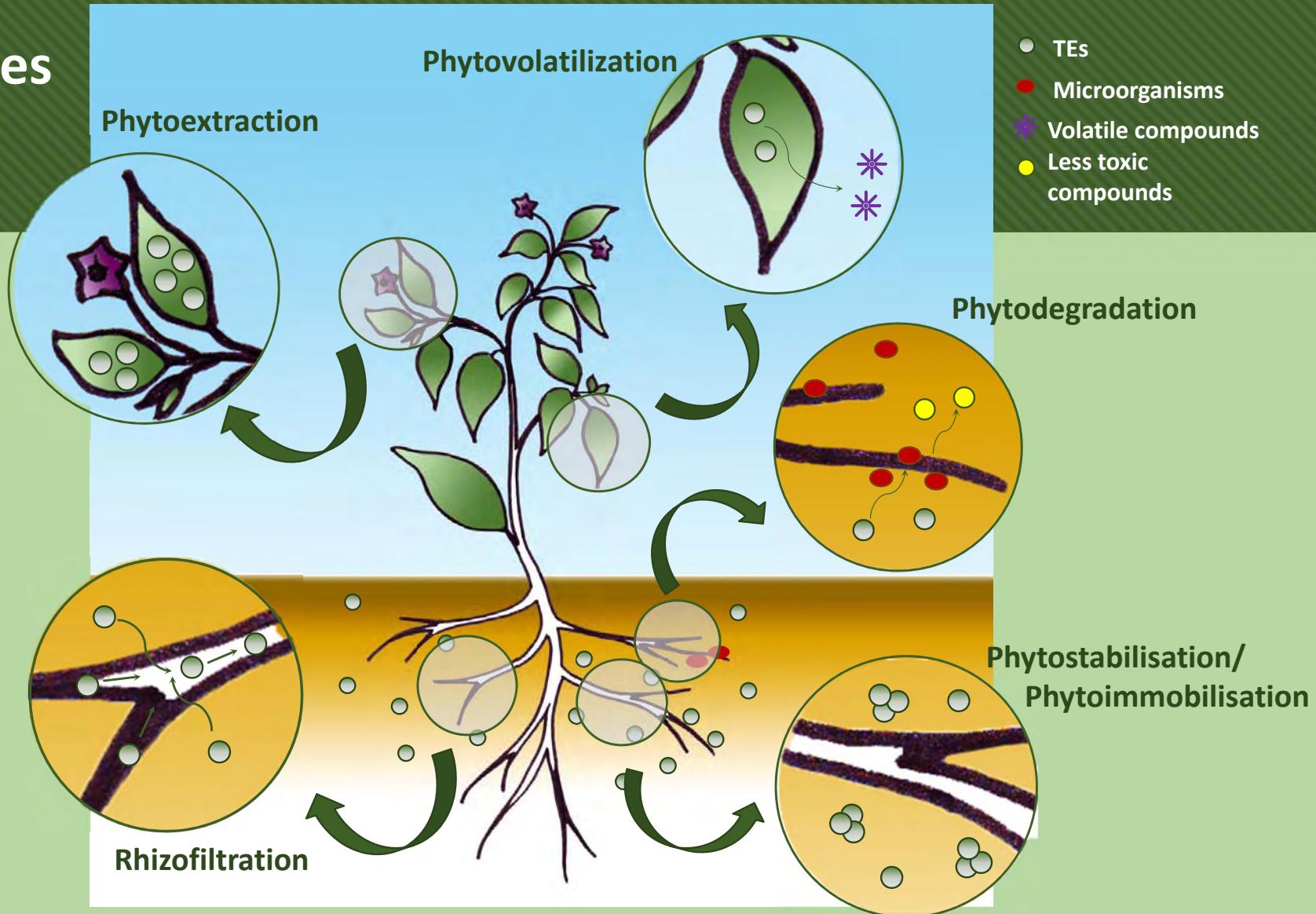


Remediation methods for contaminated soils

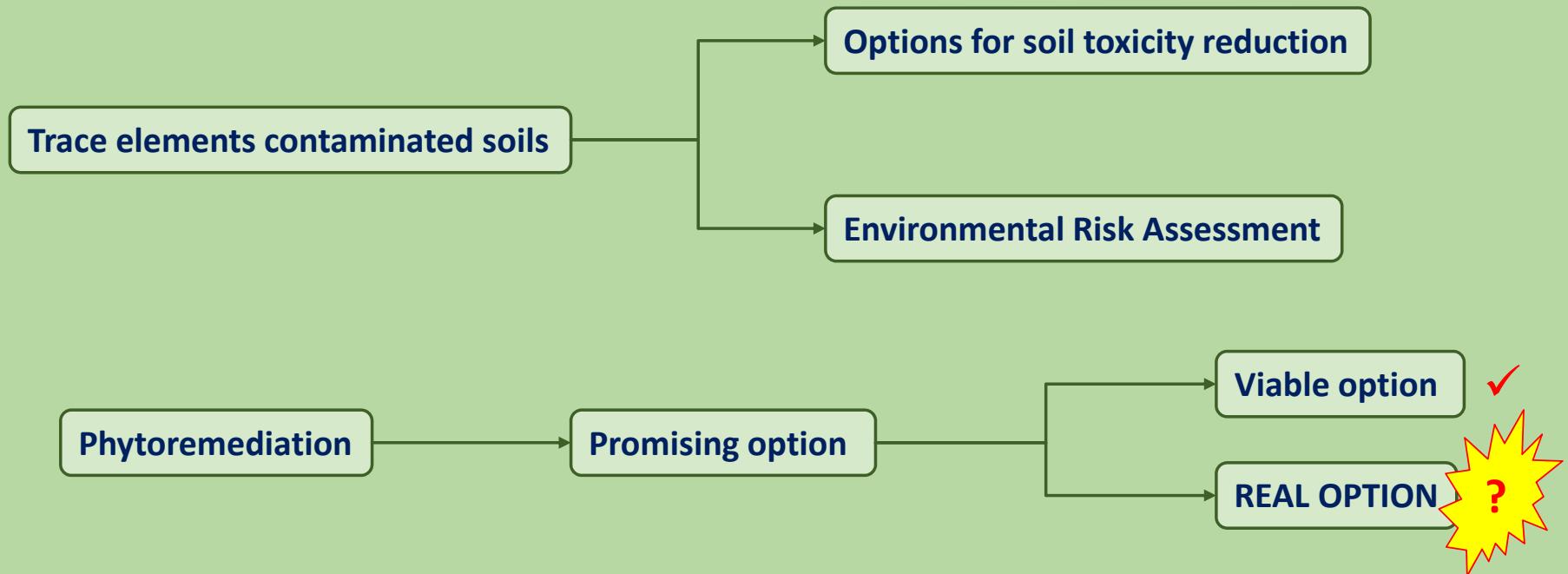


"techniques based on the use of plant species (and their associated microorganisms), **amendments** and agronomical techniques to eliminate, retain or diminish the toxicity of the contaminants" (Salt et al., 1998; Mench et al., 2009)

Phytotechnologies



Trace elements - Contamination - Phytoremediation



Trace elements - Contamination - Phytoremediation

MAJOR CHALLENGES

1. Conditioning of the soils
 - Acidity/alkalinity, fertility, structure
2. Plant survival & growth: species selection
 - Tolerance, adapted, accumulation/exclusion
3. Monitoring and assessment
 - Eco-toxicity, bioindicators, ecosystem services
4. Management of the generated biomass
 - Valorization options

Conditioning of the soils



Soil amelioration and conditioning

Waste materials: organic and inorganic

Recycling & reutilization

End of waste

Circular economy

Amendments selection: crucial

Allow/Improve plants growth

TEs solubility, mobility, speciation & bioavailability

Immobilization

↓ solubility/availability

Combination of amendments

Extraction

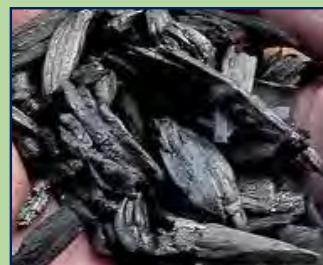
↑ solubility/availability

Acid, fresh/redox active

Conditioning of the soils

ORGANIC AMENDMENTS

- ✓ Sewage sludge
- ✓ Municipal waste
- ✓ Manure
- ✓ Compost
- ✓ Agri-food byproducts
- ✓ Biochar
- ✓ Digestate



INORGANIC AMENDMENTS

- ✓ Lime
- ✓ Zeolites
- ✓ Fe rich materials
- ✓ Nanoparticles
- ✓ Red mud
- ✓ Other residues (paper mill)

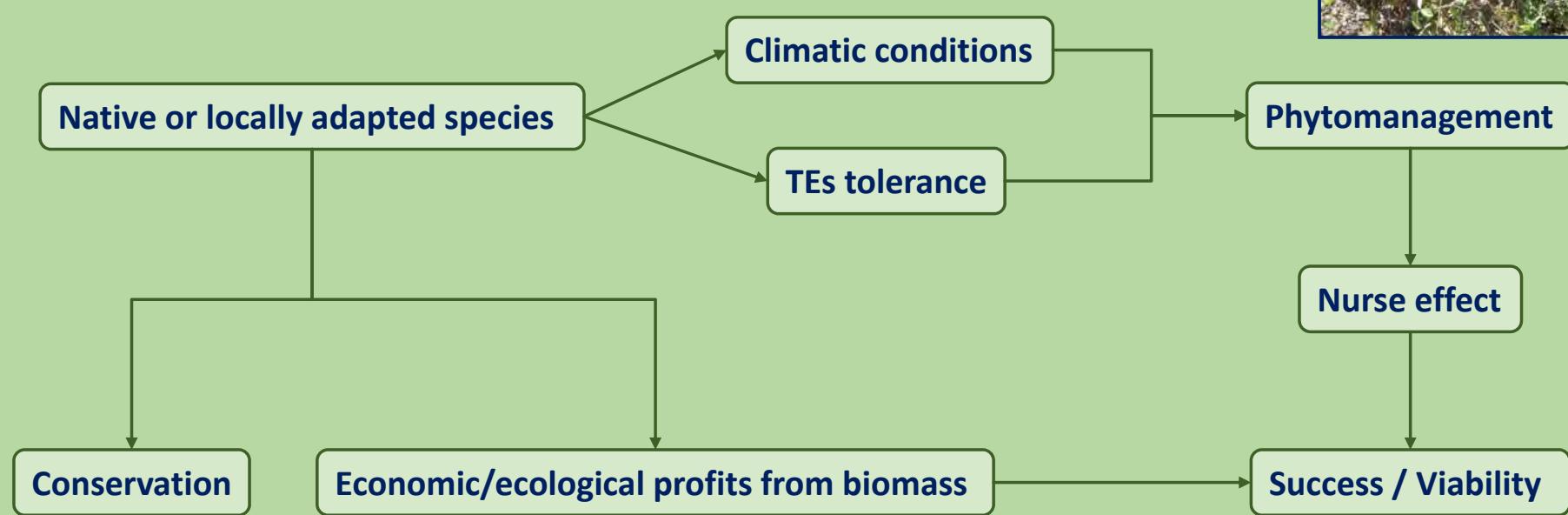


Trace elements - Contamination - Phytoremediation

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Selection of the plants



Selection of the plants: extraction vs stabilisation



Pteris vittata L. (As accumulator;
Cao et al., 2003)



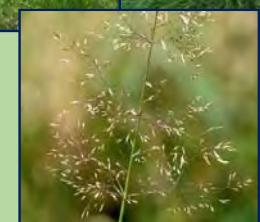
Nocaea caerulesens (Cd & Zn
hyperaccumulator; Baker et al., 1994)



Alyssum murale (Ni & Cd
phytomining; Chaney et al., 1999)



Agrostis capilaris, Festuca rubra
(Vangronsveld et al., 1998)



Soya, rapeseed,
sunflower, sorghum,
common cane
(economic revenue)



Salix spp. (willow) y *Populus*
spp. (poplar) (high biomass,
fast growth, energy crops)



Selection of the plants: native/adapted species



Dittrichia viscosa (L.) Greuter



Nicotiana glauca Graham



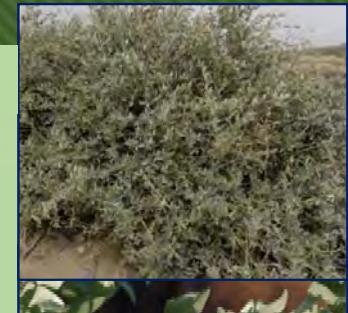
Silybum marianum (L.) Gaertn



Atriplex halimus (L.)



Bituminaria bituminosa (L.)
Stirton



Piptatherum miliaceum (L.)
Cosson



Trace elements - Contamination - Phytoremediation

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Monitoring and assessment

SOIL HEALTH

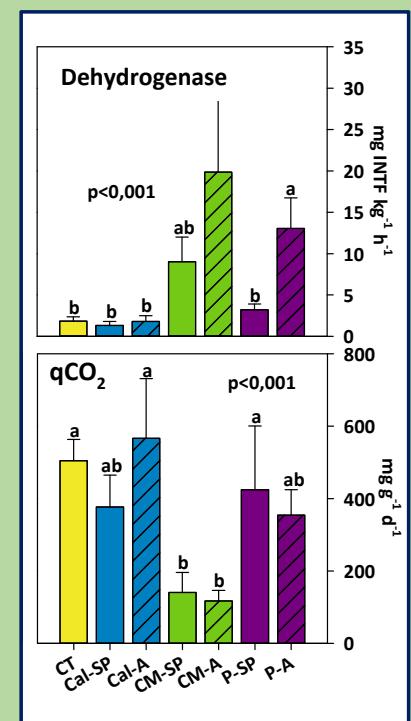
Indicators to monitor soil restoration:

- Trace elements solubility
- Soil solution (pore water)
- Accumulation in the plants



Bio-indicators (microbial parameters):

- ✓ Microbial community size ($\text{CO}_2\text{-C}$, Biomass-C, qCO_2)
- ✓ Biological functionality, general activity and functional diversity (enzymatic activities, FDA, CLPPs, etc.)

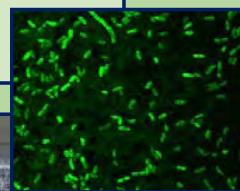


Monitoring and assessment

SOIL HEALTH

Eco-toxicity Tests (bio-assays):

- ✓ Direct (earthworms (*Eisenia fetida*) mortality, plant growth test)
- ✓ Indirect (*Vibrio fischeri* luminescence inhibition, *Daphnia magna/Thamnocephalus platyurus* immobilization, germination indices)

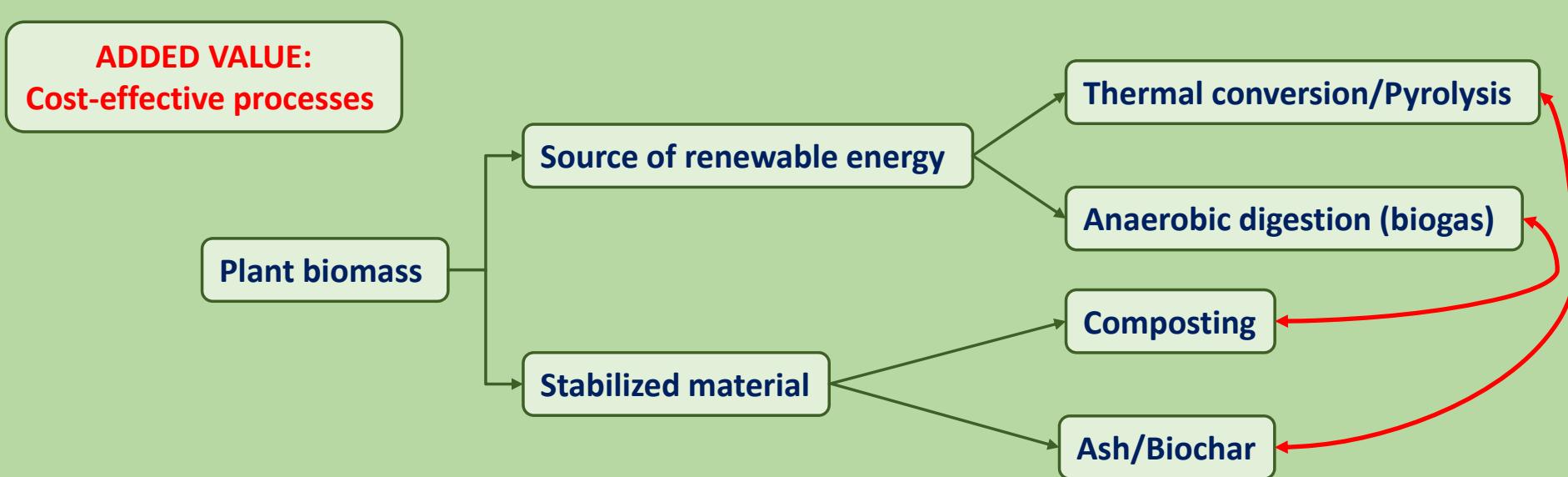


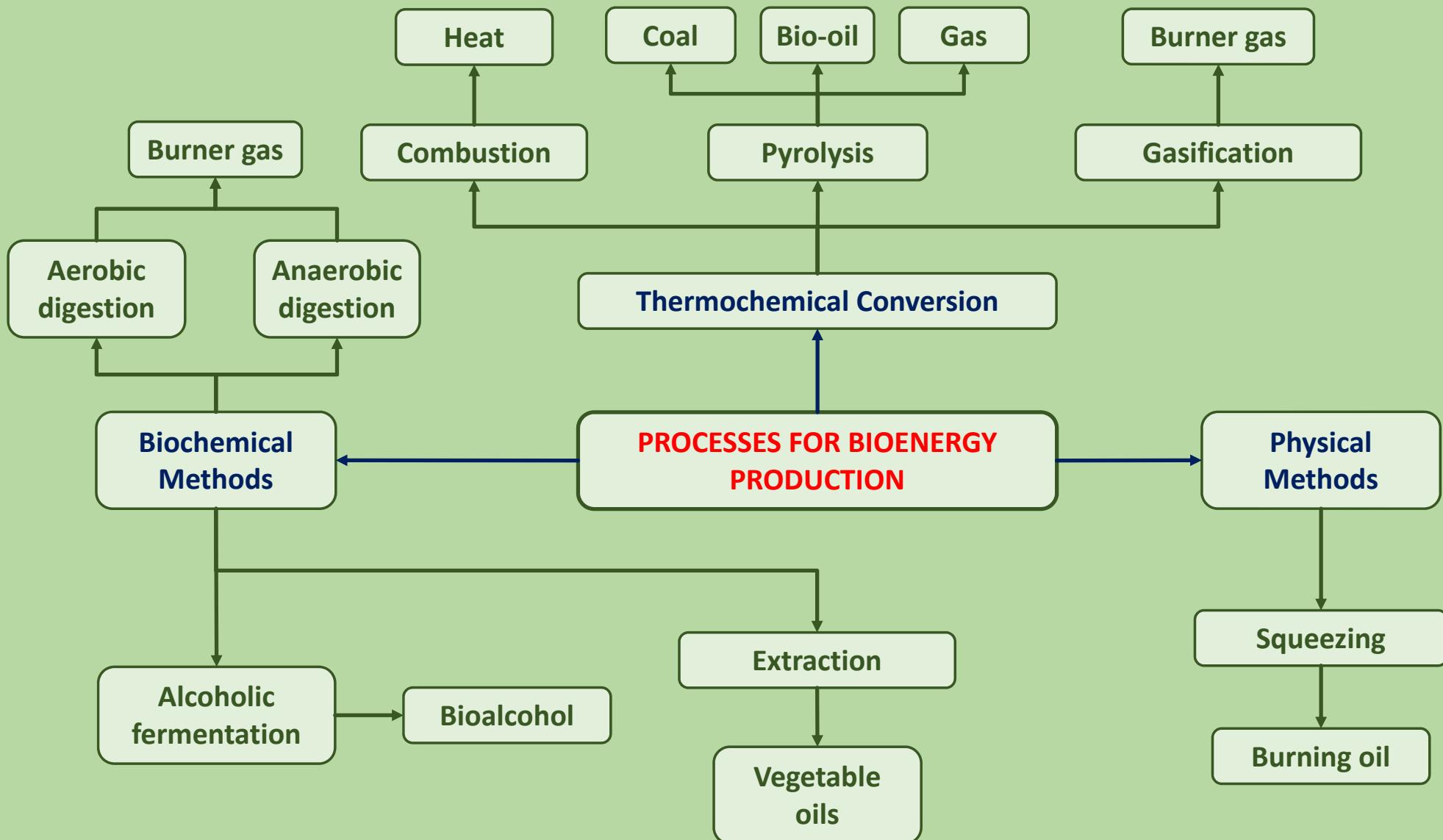
Trace elements - Contamination - Phytoremediation

MAJOR CHALLENGES

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Management of the generated biomass





Management of the generated biomass

Biomass from phytoremediation

- ✓ Potential use as energy crops
- ✓ Degradability and biogas generation potential (ignocellulosic components/carbohydrate rich, high content of biodegradable organic matter)
- ✓ Biomass thermic potential (low water and ashes content; high amount of raw lignin)
- ✓ TEs effects on biomass energetic potential

Other advantages

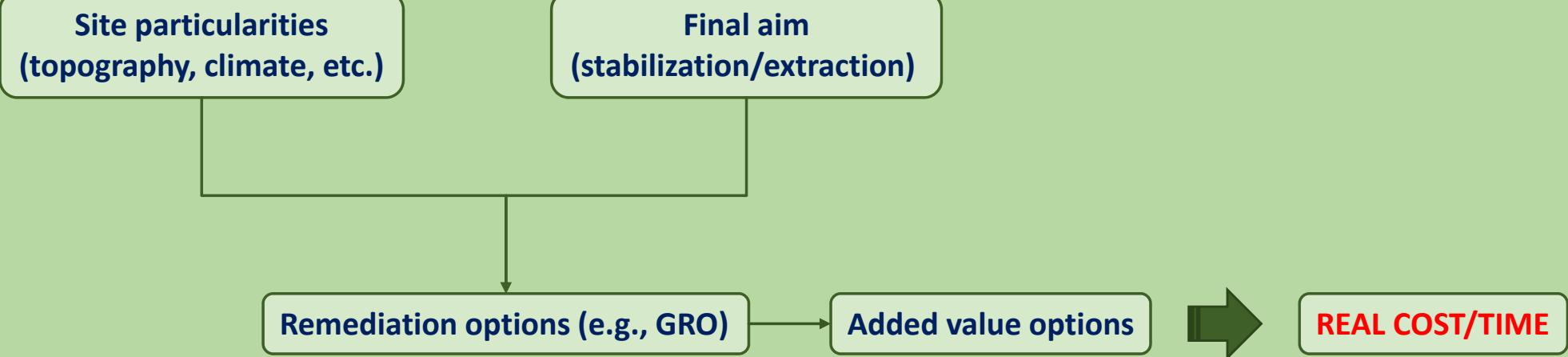
Impact on agricultural soil

- ✓ (Indirect) Land use change
- ✓ Bio-fuel life cycle emissions ↓

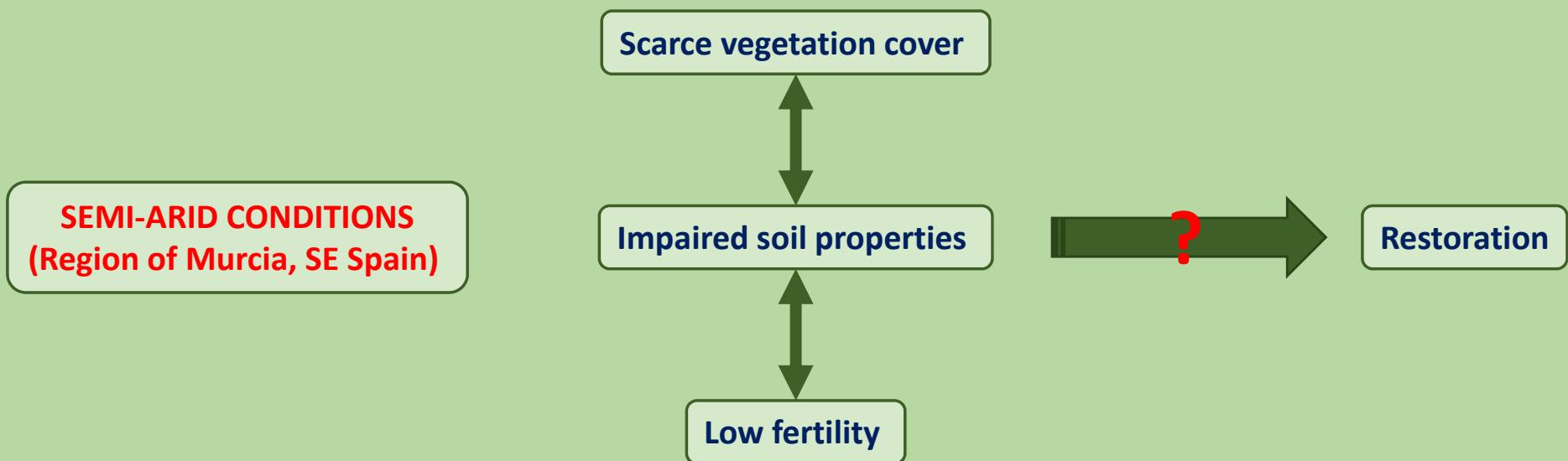


Trace elements - Contamination - Phytoremediation

**MAJOR NEED:
IMPLEMENTATION/PUT INTO PRACTICE**



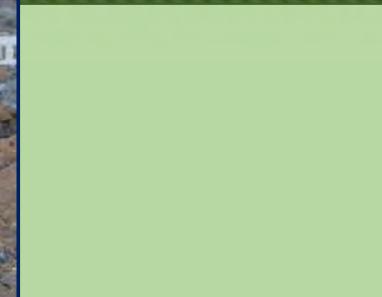
Trace elements - Contamination - Phytoremediation



La Unión-Cartagena Mining Area (Murcia, Spain)



La Unión-Cartagena Mining Area (Murcia, Spain)



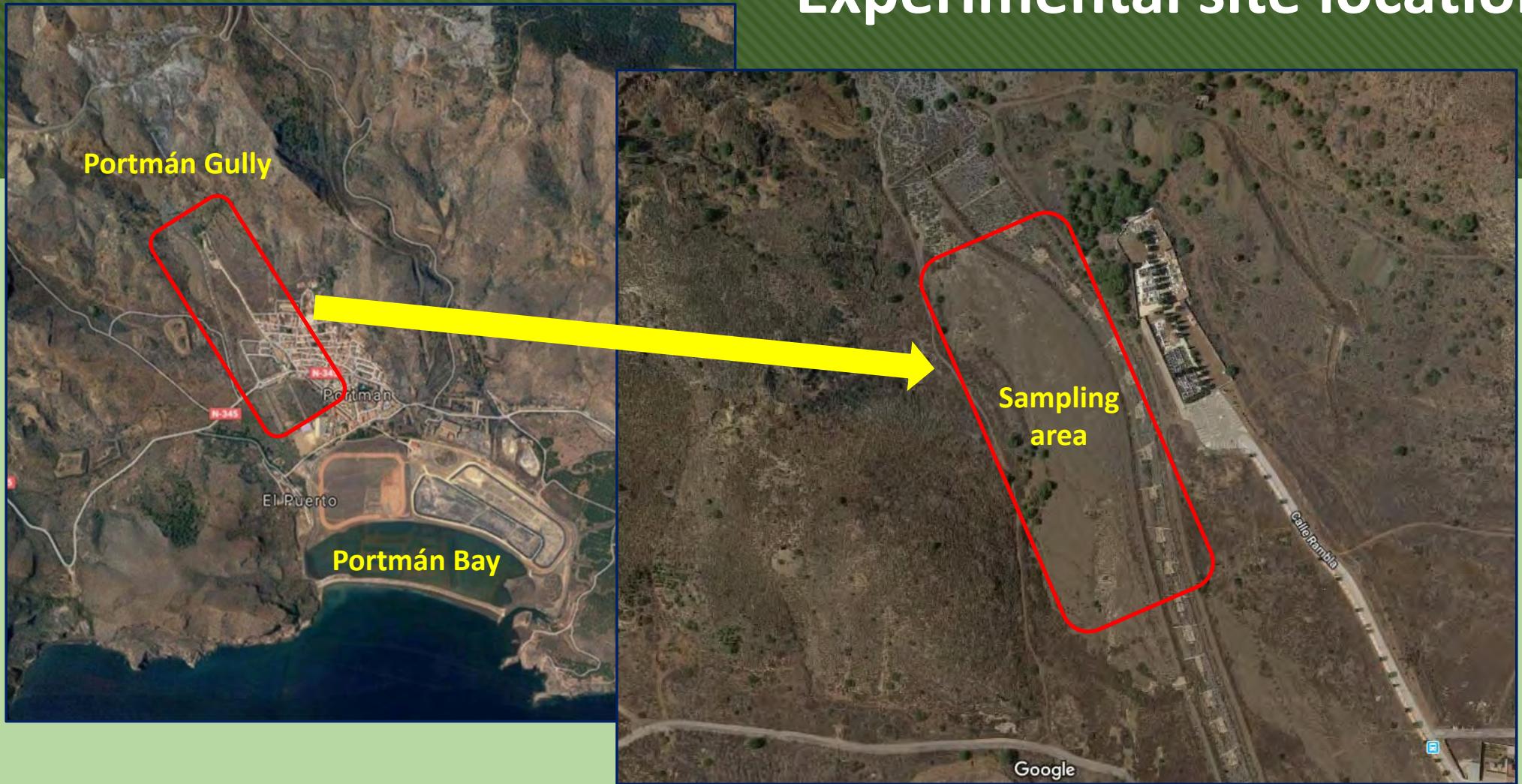
Case study 1 - Options for mine site restoration

- 1. Soil survey within the mining area of La Unión-Cartagena (Murcia, Spain)**
- 2. Selection of amendments**
- 3. Plant species: adapted, added value**
- 4. Pot test: performance of plants and amendments**
- 5. Upscale: biomass production, eco-toxicity**

Experimental site location



Experimental site location

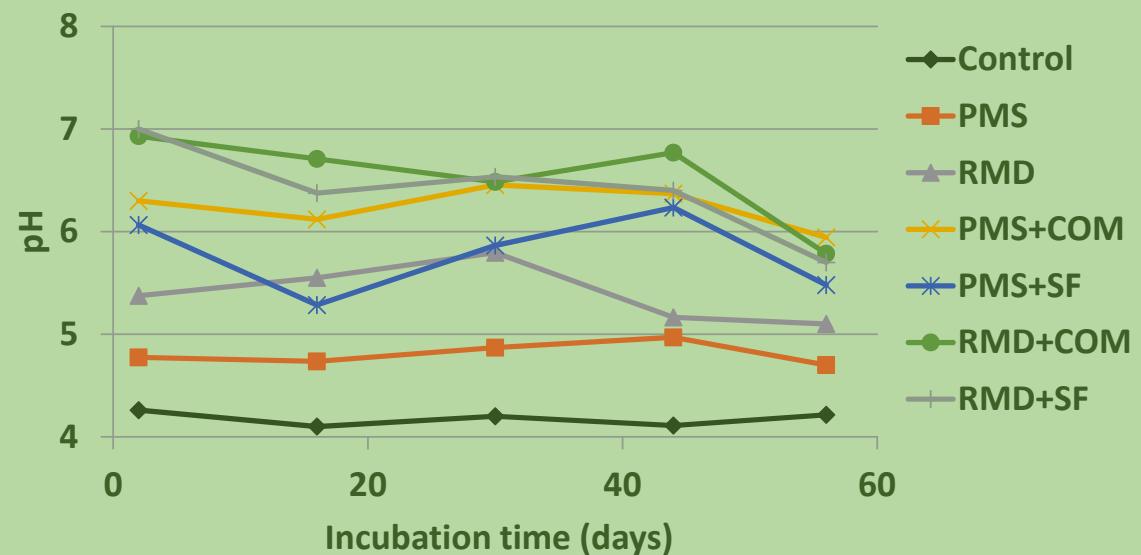




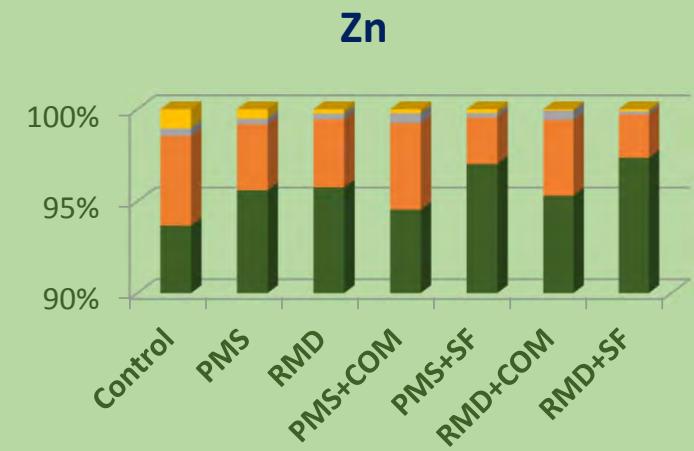
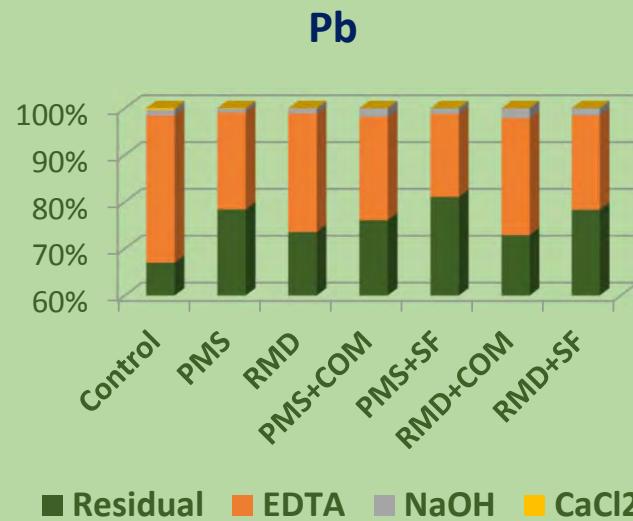
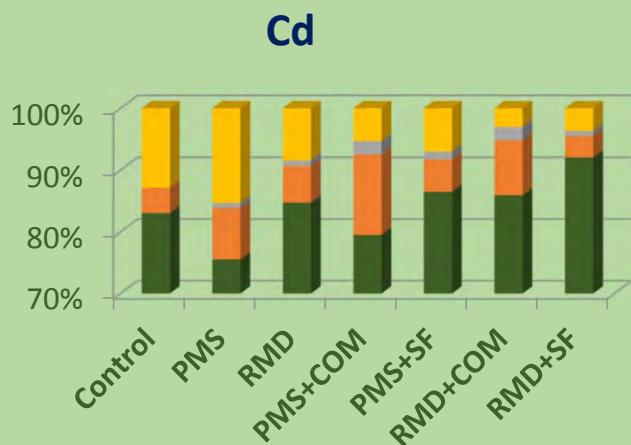
Soil characterisation and conditioning

	Portmán soil	$\text{CaCl}_2/\text{NaHCO}_3$
pH	4.16	-
TOC (g kg^{-1})	2.7	-
EC (mS cm^{-1})	2.22	-
As (mg kg^{-1})	1976	0.67
Cd (mg kg^{-1})	12	0.2
Cu (mg kg^{-1})	230	1.6
Fe (mg kg^{-1})	214747	0.5
Mn (mg kg^{-1})	968	9.3
Pb (mg kg^{-1})	19129	1667
Zn (mg kg^{-1})	2257	28

Paper mill sludge (PMS) - Red mud derivative (RMD)
Pig slurry (solid fraction, SF) - Compost (olive mil waste, COM)

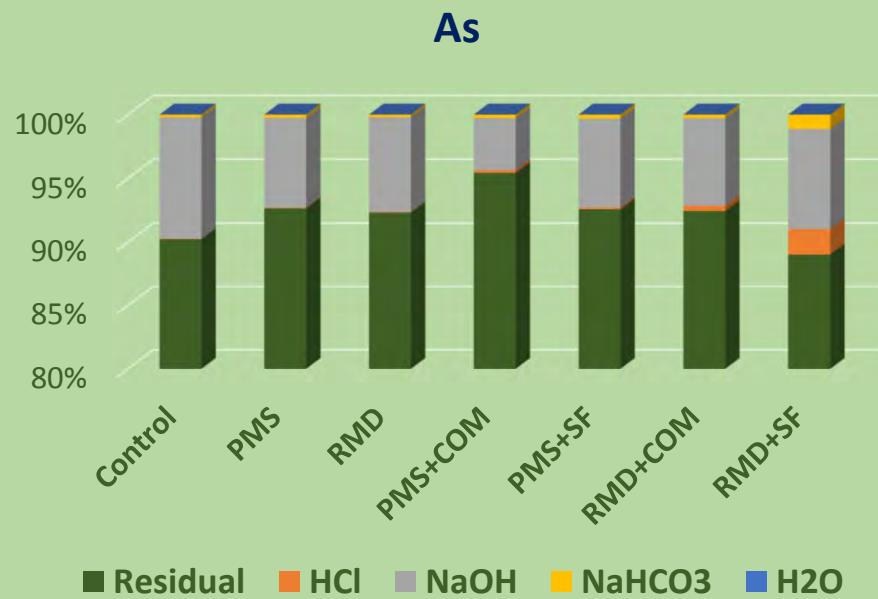


Sequential extractions



■ Residual ■ EDTA ■ NaOH ■ CaCl₂

Sequential extractions



Plants test



Plants test



- ✓ *Cynara cardunculus* ↓
- ✓ *Silybum marianum* ↑
- ✓ *Nicotiana glauca* ↑
- ✓ *Limonium caesium* ↓
- ✓ *Piptatherum miliaceum* ↑

Pot test

Soil and amendments

	Soil	SF	PMS	RMD
pH	3.80	7.31	8.87	10.9
EC (dS m ⁻¹)	0.19	6.85	4.40	0.14
OM (%)	0.28	68.6	32.2	n.d.
TOC (g kg ⁻¹)	1.63	356	195	7.17
TN (g kg ⁻¹)	0.31	32.8	20.0	0.15
P (g kg ⁻¹)	0.29	7.7	0.26	0.37
As (mg kg ⁻¹)	1976	<0.1	<0.1	4.68
Cd (mg kg ⁻¹)	12.0	0.2	0.19	0.27
Cu (mg kg ⁻¹)	230	226	184	55.7
Fe (g kg ⁻¹)	215	1.36	9.10	90.2
Mn (mg kg ⁻¹)	968	194	72.7	901
Pb (mg kg ⁻¹)	19129	5.3	18.4	41.2
Zn (mg kg ⁻¹)	2257	849	38.6	10.7

n.d.: not determined.

- ✓ Pig slurry (solid fraction, SF)
- ✓ Paper mill sludge (PMS)
- ✓ Red mud derivative (RMD)

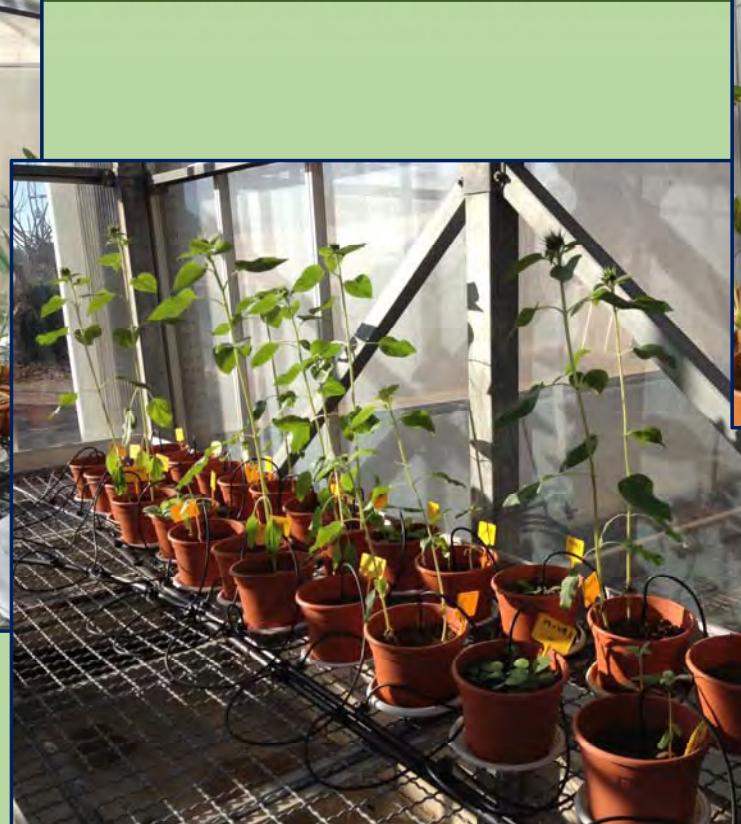
Pot test

S. Marianum
P. miliaceum



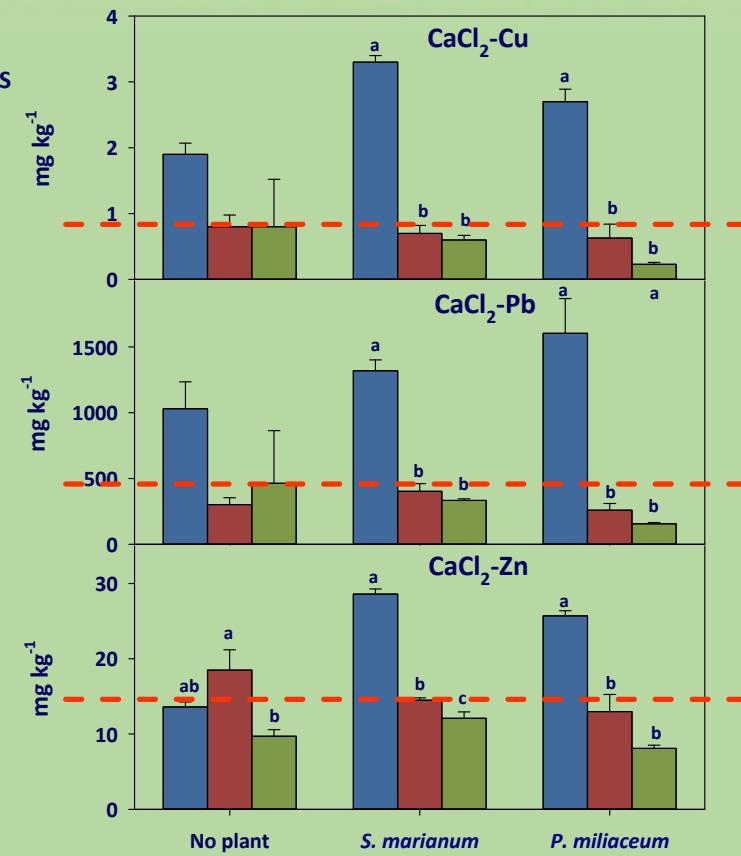
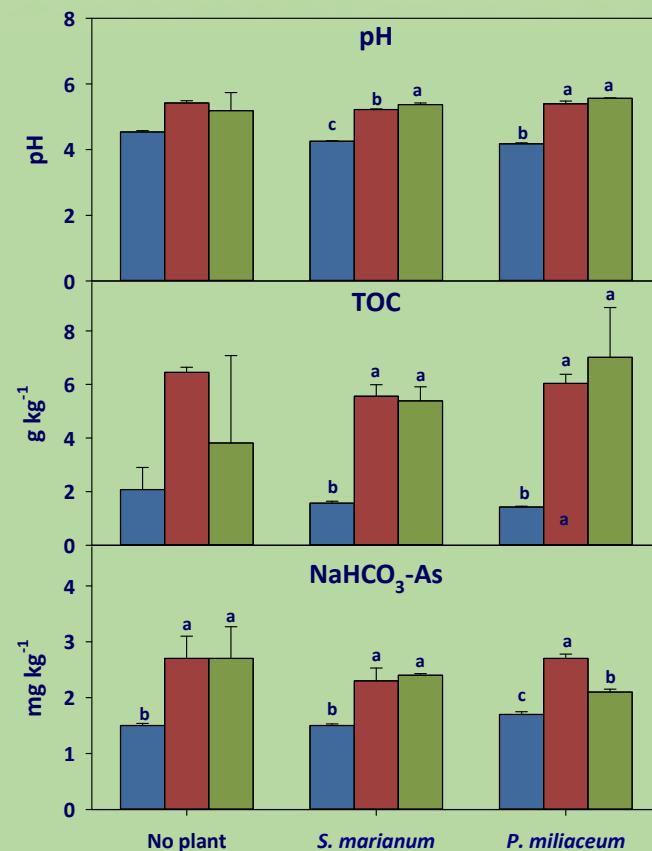
Pot test

N. glauca
H. annuus



Pot test Effects on soils

pH, TOC,
Metals & As extractability



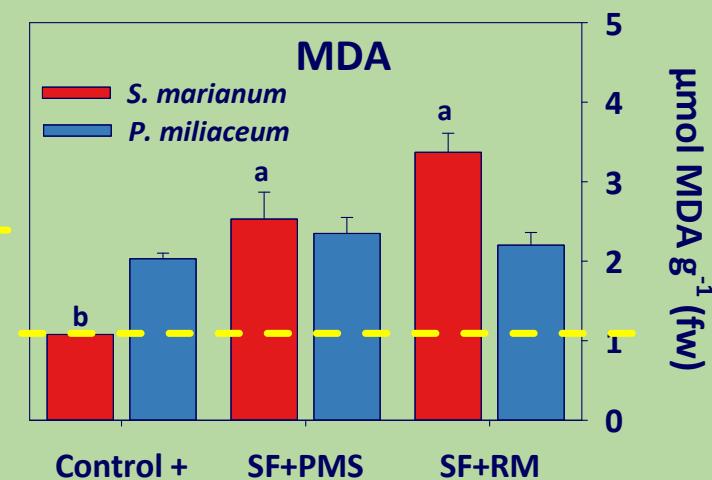
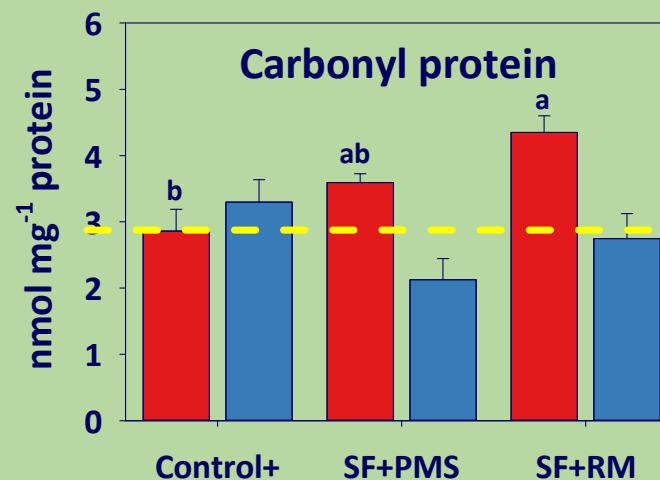
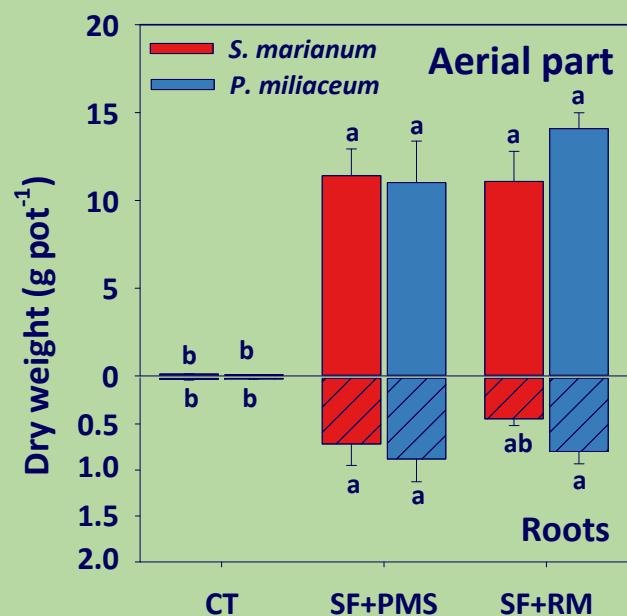
Pot test Plants – TEs accumulation

TE CONCENTRATIONS (mg kg⁻¹ dw) IN THE PLANTS (mean ± se)

	As	Cd	Cr	Cu	Pb	Zn	Mn
<i>Piptatherum miliaceum</i>							
RM	7.3 ± 1.20	0.05 ± 0.10	63.6 ± 9.5ab	20.0 ± 1.0b	91 ± 3.0	124 ± 2.5cd	211 ± 8.0d
PMS	8.4 ± 2.45	0.03 ± 0.02	25.1 ± 4.4b	19.0 ± 1.2b	114 ± 24.5	99 ± 0.1d	168 ± 10.0cd
<i>Silybum marianum</i>							
RM	2.5 ± 0.60	1.95 ± 0.15	73.5 ± 8.5ab	21.3 ± 0.8b	118 ± 18.5	293 ± 17.0b	639 ± 4.0a
PMS	3.2 ± 0.90	2.50 ± 0.50	123.0 ± 40a	27.8 ± 0.6a	183 ± 10.5	407 ± 12.5a	577 ± 6.5b
<i>Helianthus annuus</i>							
RM	0.6 ± 0.52	0.45 ± 0.05	12.4 ± 0.91b	6.3 ± 0.4d	87 ± 10.0	100 ± 7.0d	229 ± 22.5c
PMS	1.2 ± 0.15	0.59 ± 0.05	24.7 ± 5.20b	7.9 ± 1.5d	161 ± 5.5	145 ± 6.9c	239 ± 8.0c
<i>Nicotiana glauca</i>							
RM	2.6 ± 1.76	1.50 ± 0.06	21.8 ± 2.48b	10.0 ± 1.1c	225 ± 6.5	137 ± 9.5c	72.5 ± 12.0e
PMS	1.6 ± 0.37	1.59 ± 0.36	18.1 ± 1.87b	10.1 ± 0.9c	231 ± 26	137 ± 3.0c	78.0 ± 5.0e
ANOVA							
TxP	n.s.	n.s.	*	**	n.s.	**	**

*, **: P < 0.05, and 0.01, respectively; n.s.: not significant. Post-hoc: Tukey's test at P < 0.05.

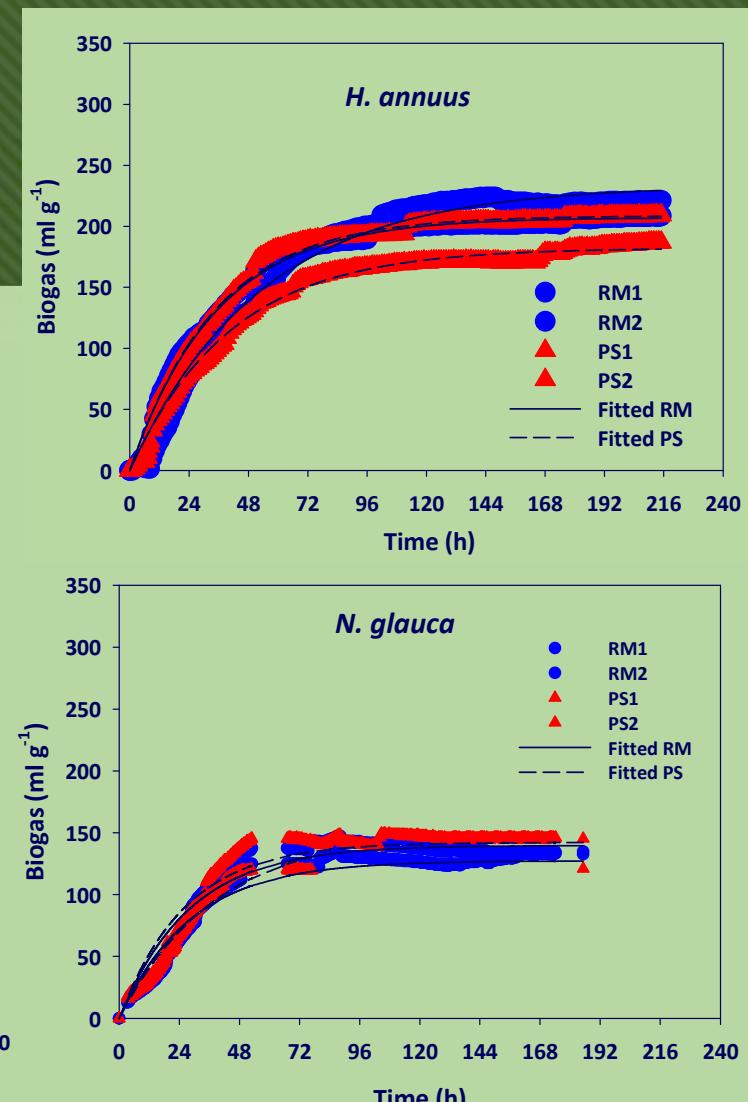
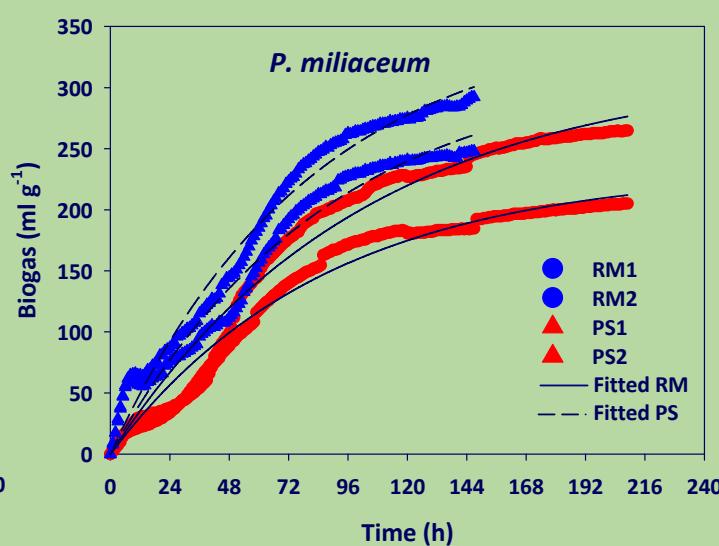
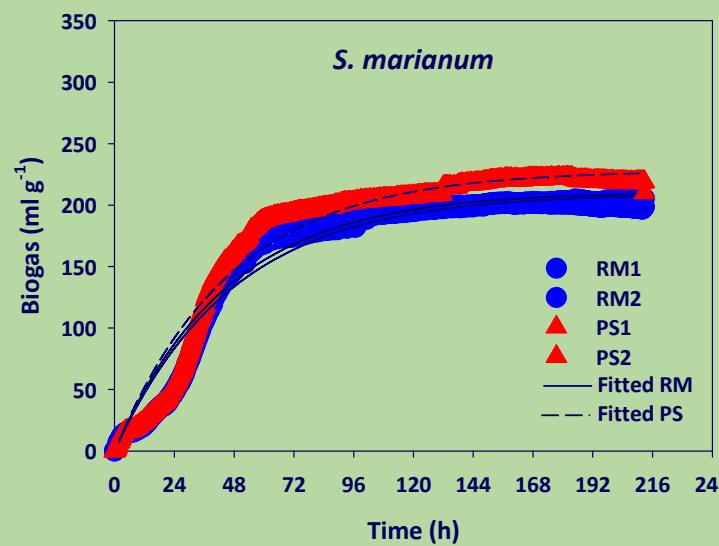
Pot test Plants - Oxidative stress



Pot test

Biomass valorisation

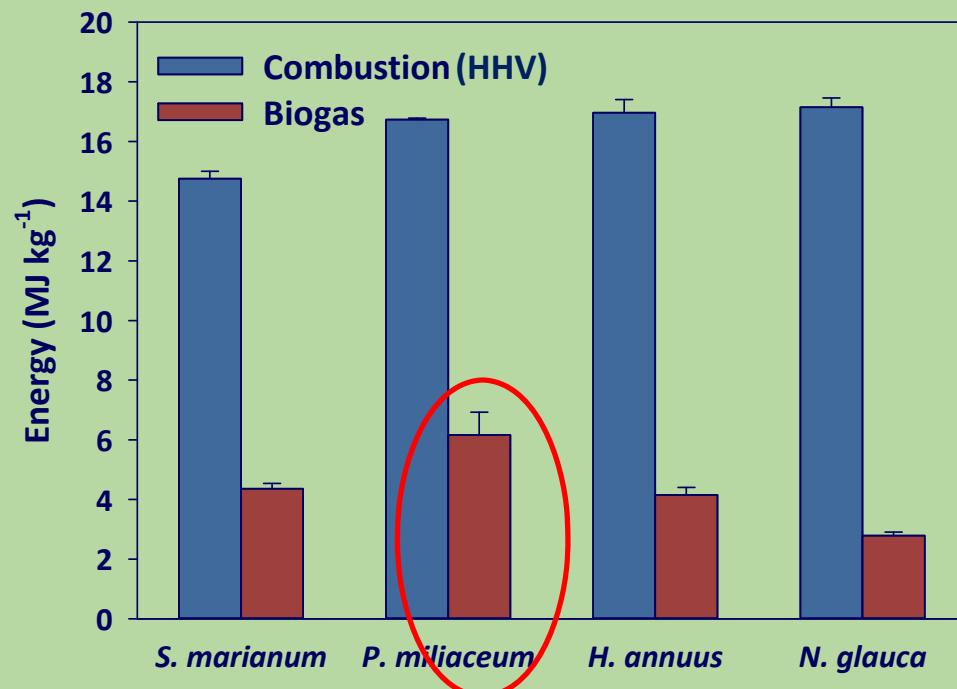
ANAEROBIC INCUBATION (ANKOM RF) - BIOGAS



Bernal et al., 2019. *Biomass and Bioenergy* 126, 220-230.

Pot test Biomass valorisation

HHV: Higher heating value (calculated from elemental composition)



Bernal et al., 2019. *Biomass and Bioenergy* 126, 220-230.

Planter test

Silybum marianum
Helianthus annuus

NON CONTAMINATED SOIL



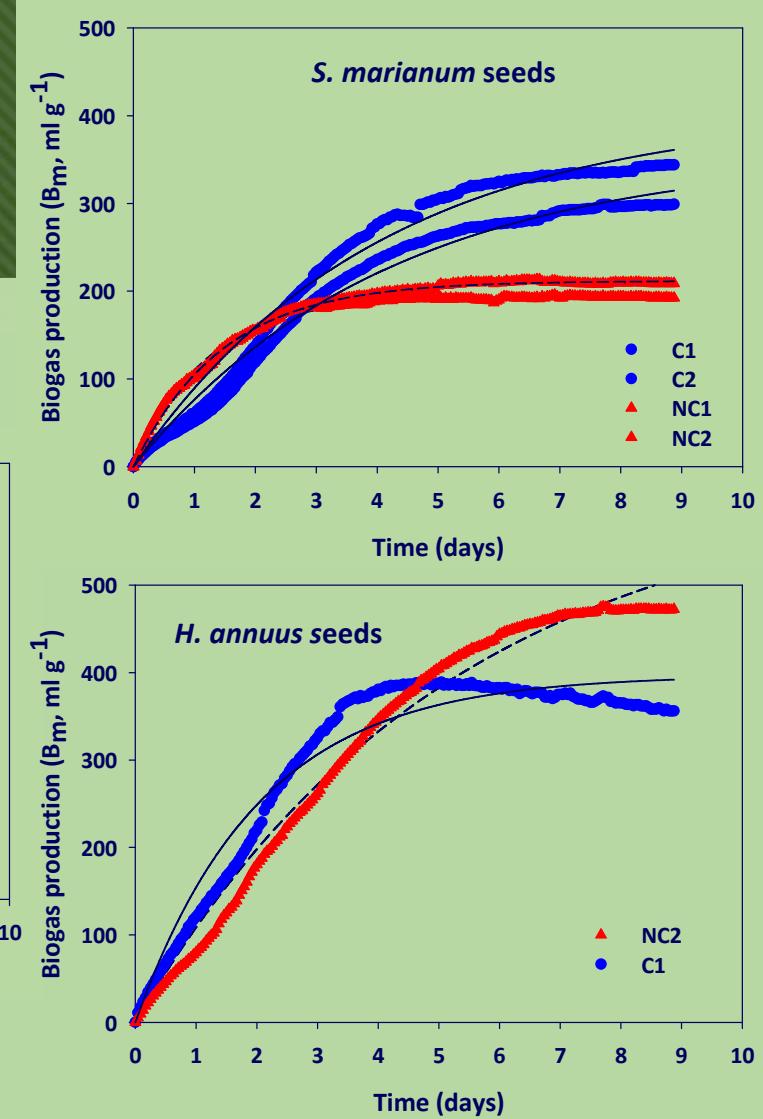
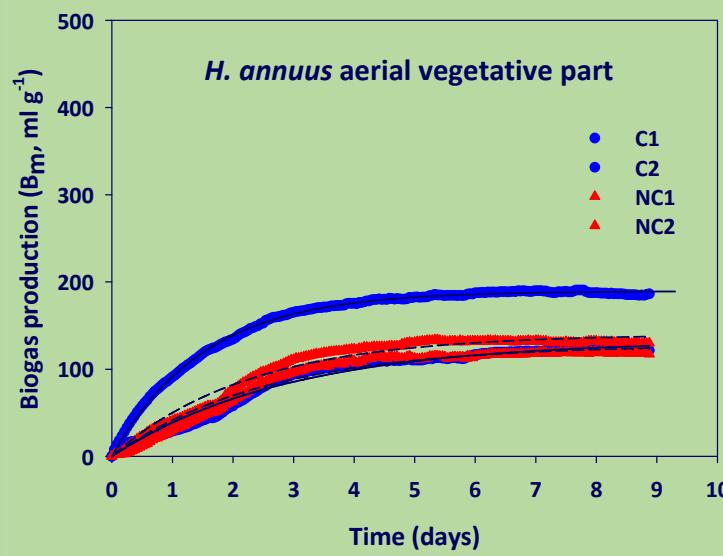
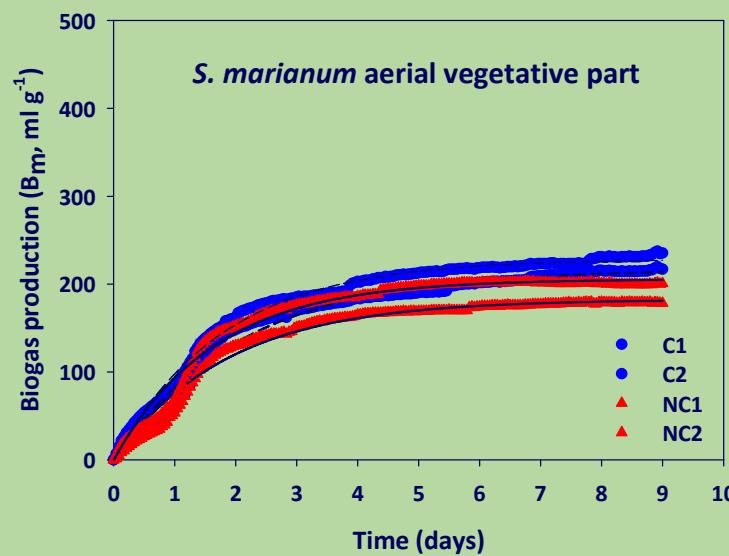
CONTAMINATED SOIL
SF + PMS



Planter test

Biomass valorisation

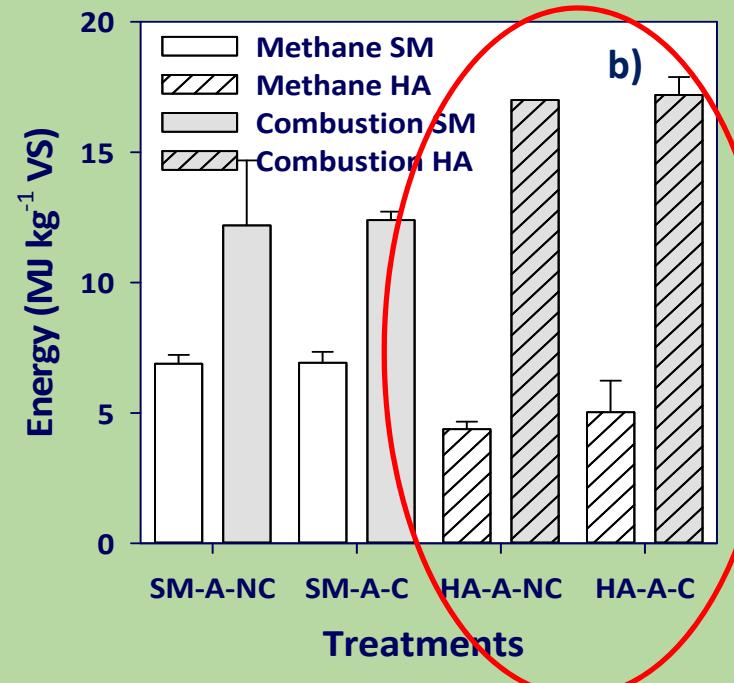
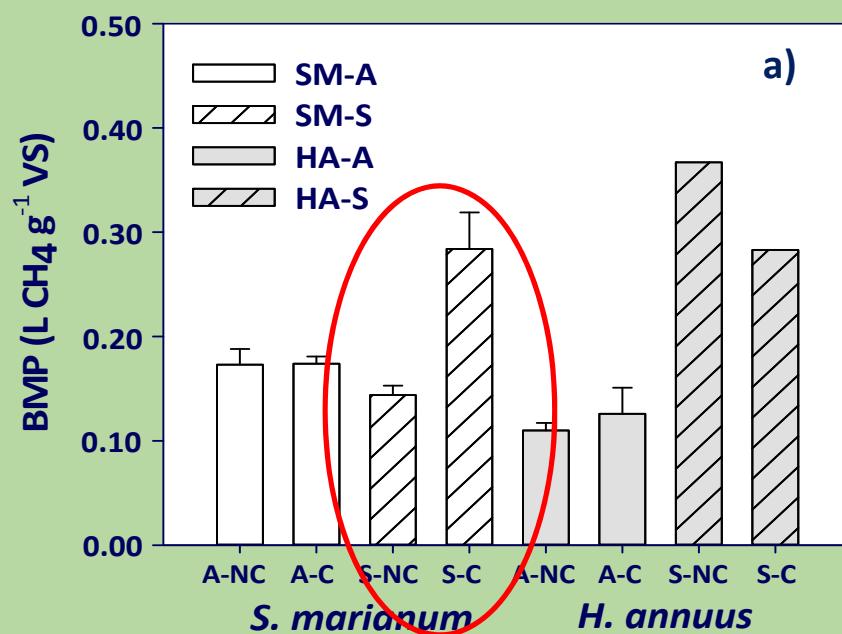
ANAEROBIC INCUBATION - BIOGAS



Planter test

Biomass valorisation

- a) Biomethane production potentials (BMP)
- b) Comparison of energy production methods: anaerobic digestion (methane) and combustion (HHV)



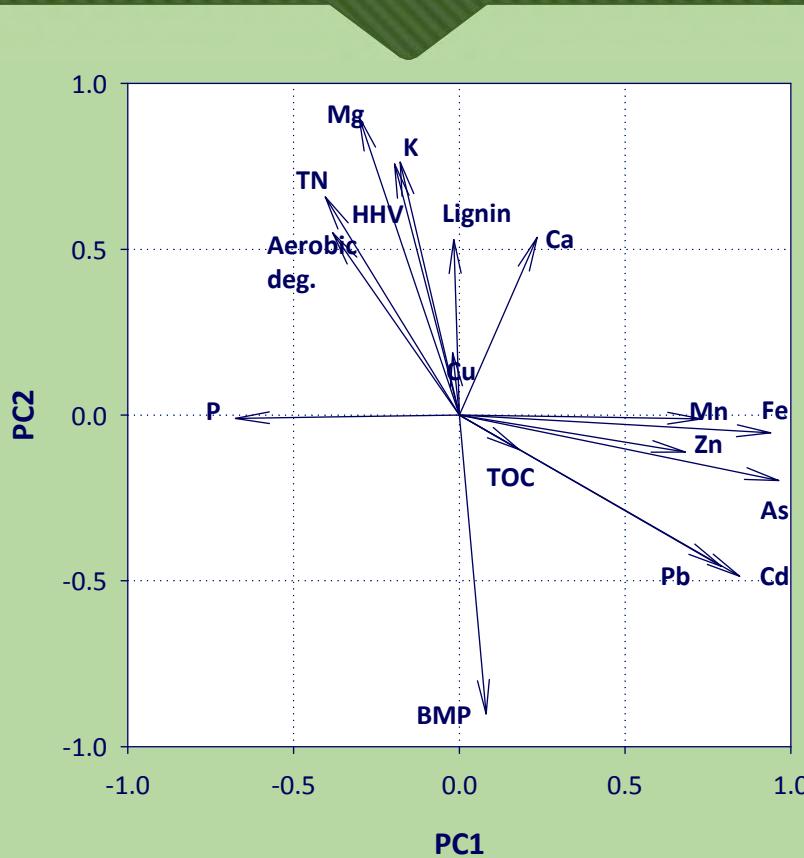
Hunce et al., 2019. *Industrial Crops & Products* 135, 206-216.

SM: *S. marianum*;
HA: *H. annuus*;

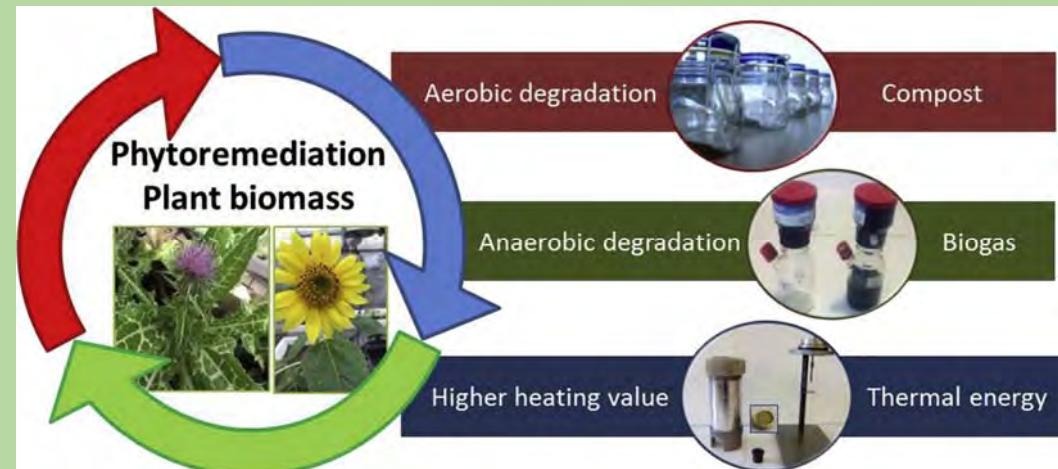
A: aerial part;
S: seeds;

NC: non-contaminated
C: contaminated

Planter test Principal Component Analysis



- ✓ BMP (inversely), HHV and aerobic degradation related to lignin and nutrient content in the plants
- ✓ Metals and As concentration do not affect degradability and energy production potential



Hunce et al., 2019. *Industrial Crops & Products* 135, 206-216.

Planter test Eco-toxicological evaluation

Plant growth test (direct) - *Helianthus annuus* & *Lactuca sativa*

Artificial soil (OCDE)

0.6 kg peat

1.6 kg clay

5.6 kg sand

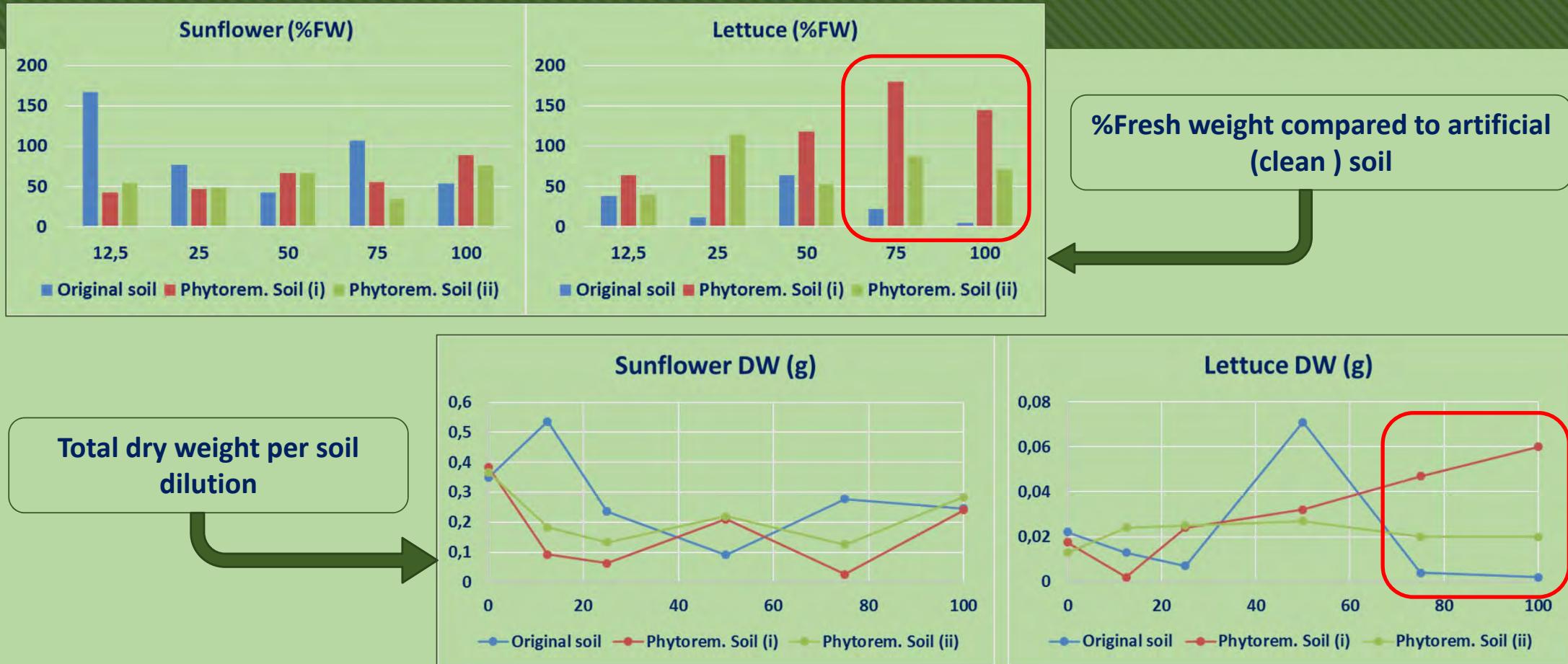
0.08 kg CaCO₃



Soil dilutions

Helianthus annuus/Lactuca sativa
cultivated in growth chamber

Planter test Eco-toxicological evaluation

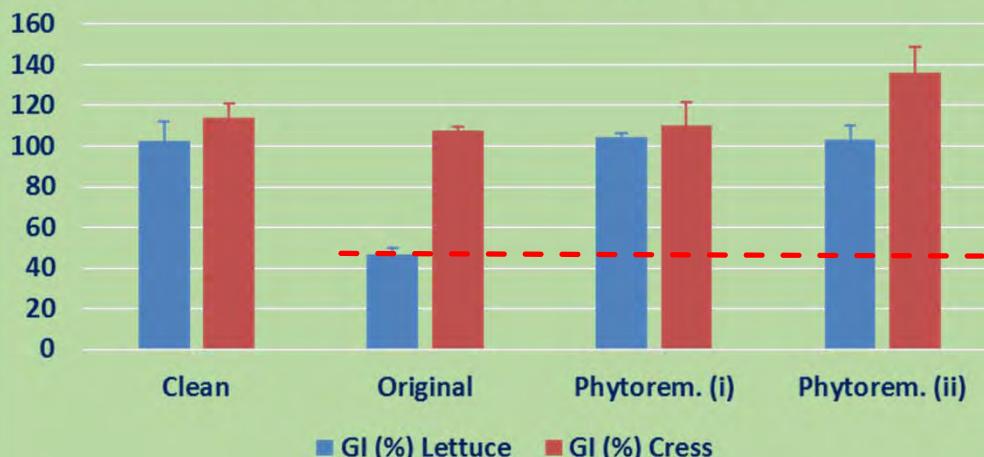


Planter test Eco-toxicological evaluation

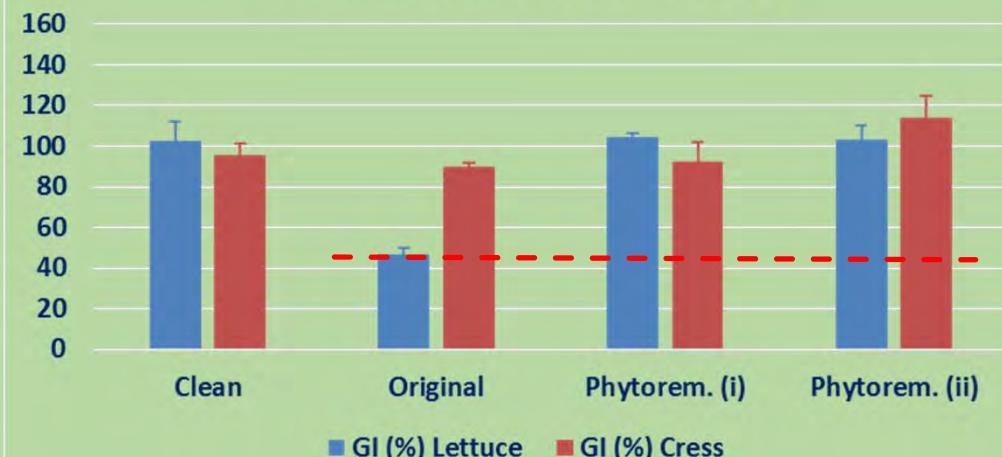
Germination index (indirect) - *Lepidium sativum* & *Lactuca sativa*



Germination index - H_2O

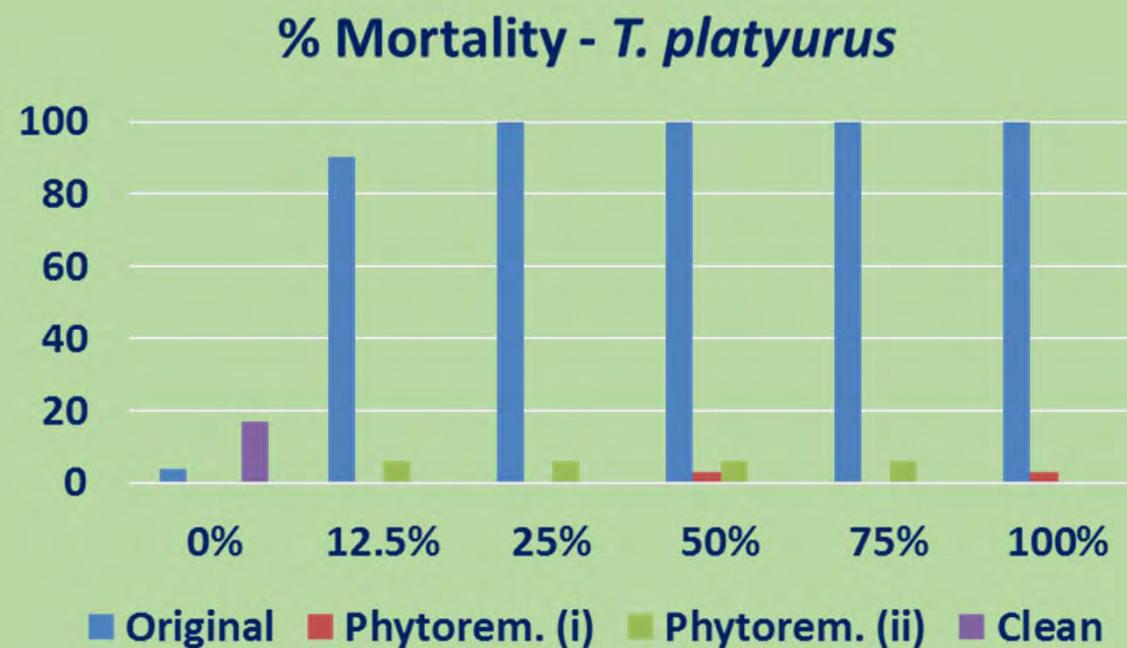


Germination index - CaCl_2



Planter test Eco-toxicological evaluation

Mortality test (indirect) – *Thamnocephalus platyurus*



Case study 1 - CONCLUSIONS

- ✓ SF + inorganic amendments **stabilized TEs** in a mine soil
- ✓ Plants TE concentrations within common ranges for mine soils
- ✓ Carbonyl proteins and MDA levels confirmed oxidative stress in plants
- ✓ Amendments-native species combination suitable for mine soils
phytostabilization
- ✓ **No effects of TEs** in the plants on bioenergy production were observed
- ✓ Combustion was the best option for **bioenergy production** from these plants
- ✓ Phytostabilized soils showed **no toxicity**: successful remediation

Case study 2 - Phytostabilization of a mine affected soil



Case study 2 - Phytostabilization of a mine affected soil



Case study 2 - Phytostabilization of a mine affected soil



	Soil LL
pH	6.18 ± 0.05
OM (%)	0.23 ± 0.02
TOC (g kg⁻¹)	1.35 ± 0.10
N _T (g kg⁻¹)	0.45 ± 0.07
Fe (g kg⁻¹)	108 ± 1
Zn (mg kg⁻¹)	9686 ± 252
Pb (mg kg⁻¹)	10,188 ± 97
Cu (mg kg⁻¹)	193 ± 8
Mn (mg kg⁻¹)	4073 ± 368
Cd (mg kg⁻¹)	19 ± 1
As (mg kg⁻¹)	664 ± 28

Case study 2 - Phytostabilization of a mine affected soil



PIG SLURRY
($60 \text{ m}^3 \text{ ha}^{-1}$)

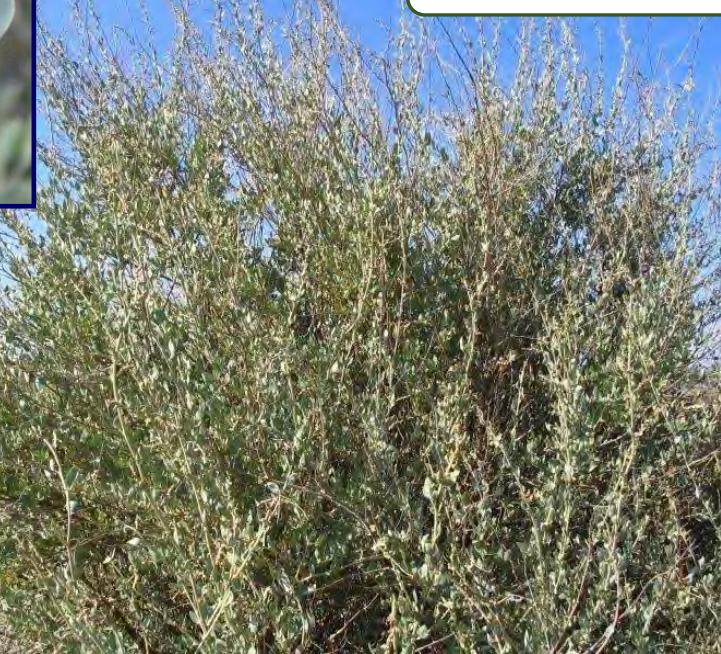
COMPOST
(60 t ha^{-1})



HYDRATED LIME
(>93% CaO)
(2.3 t ha^{-1})



Case study 2 - Phytostabilization of a mine affected soil

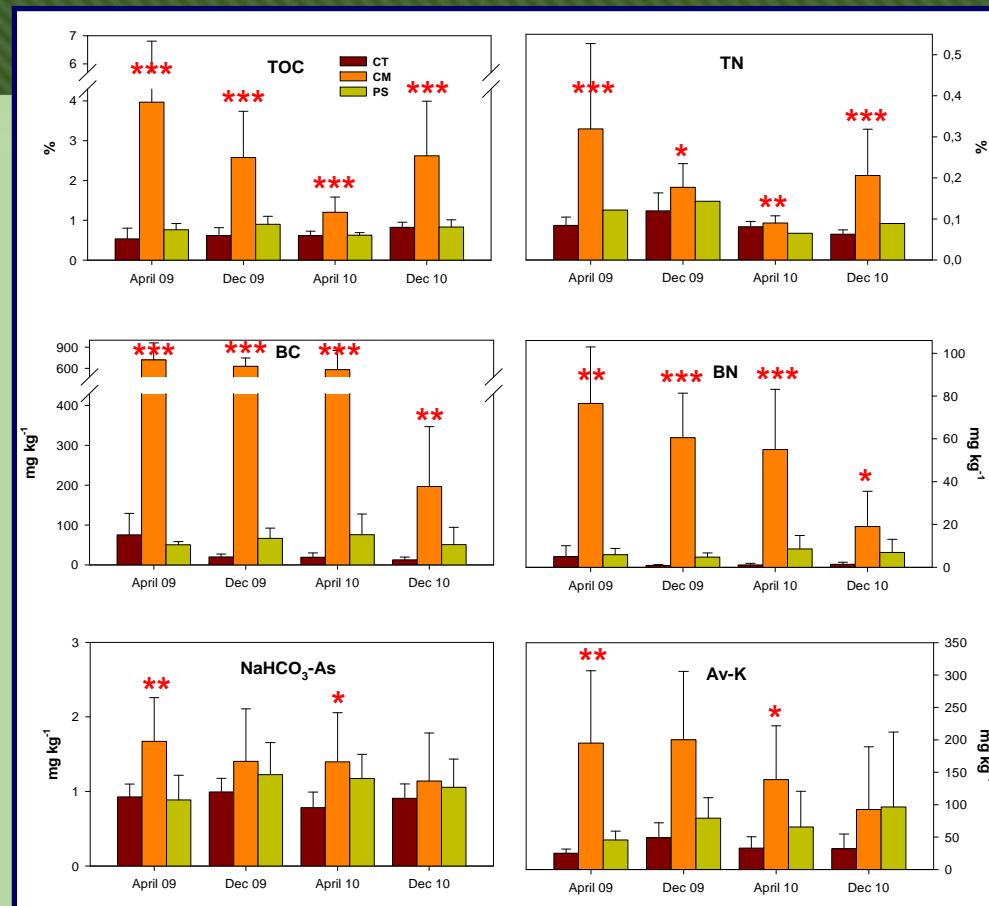


Atriplex halimus L.

Bituminaria bituminosa L.

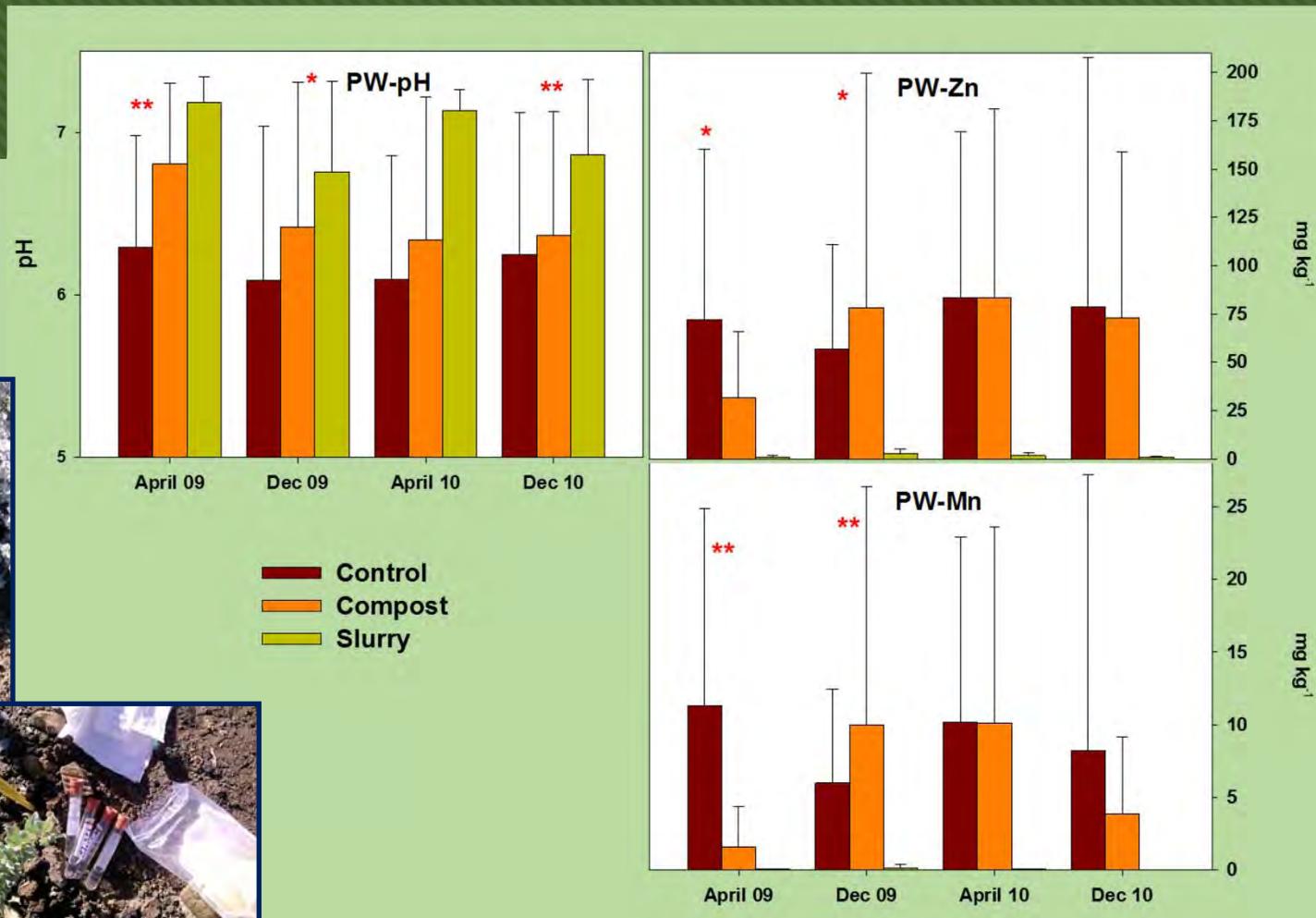


Case study 2 - SOILS



Clemente et al., 2012. *J. Hazard. Mat.* 233-234, 63-71.

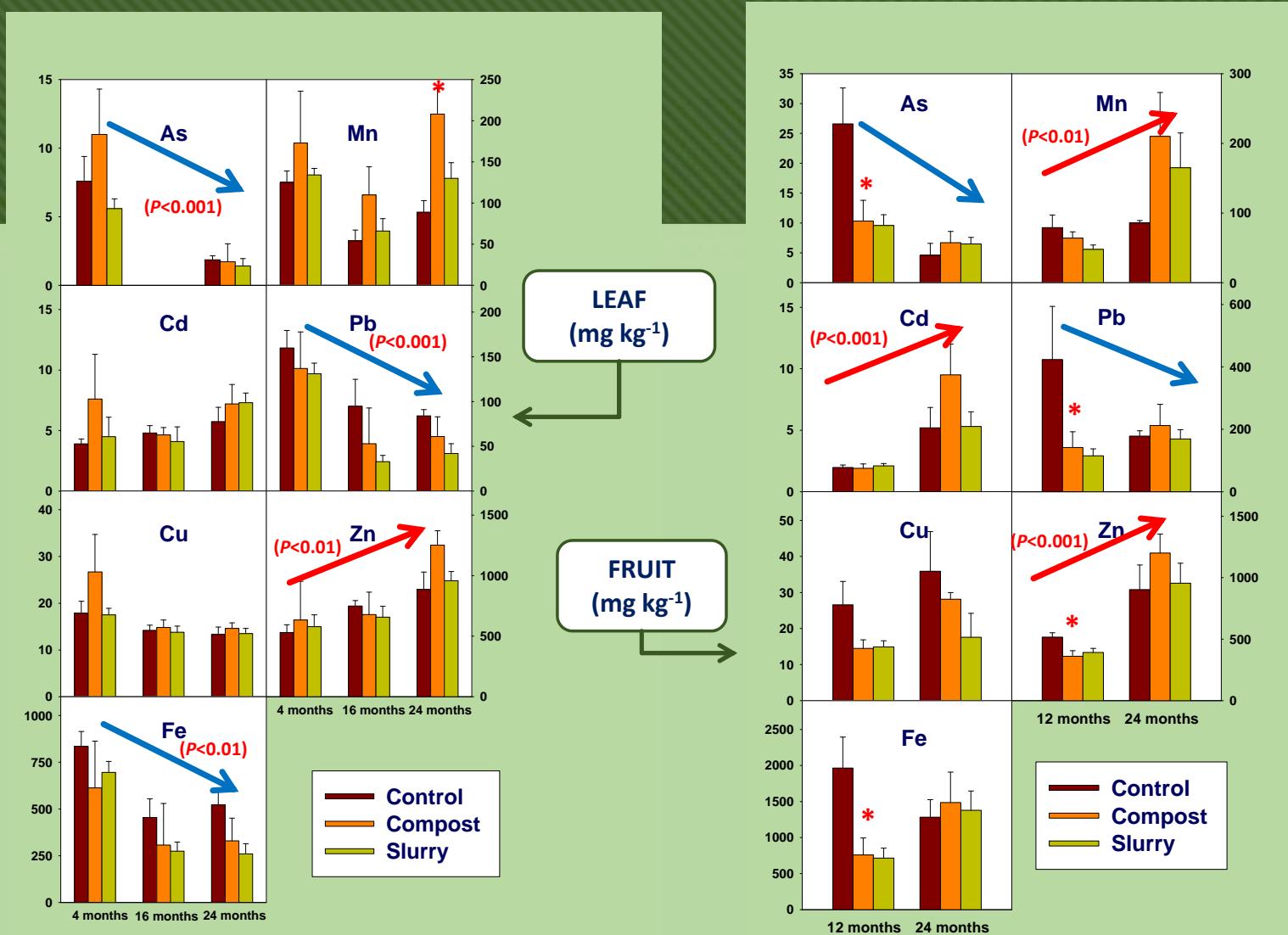
Case study 2 – PORE WATER



Clemente et al., 2012. *J. Hazard. Mat.* 233-234, 63-71.

Case study 2

PLANTS



Clemente et al., 2012. *J. Hazard. Mat.* 233-234, 63-71.

Case study 2 - PLANTS

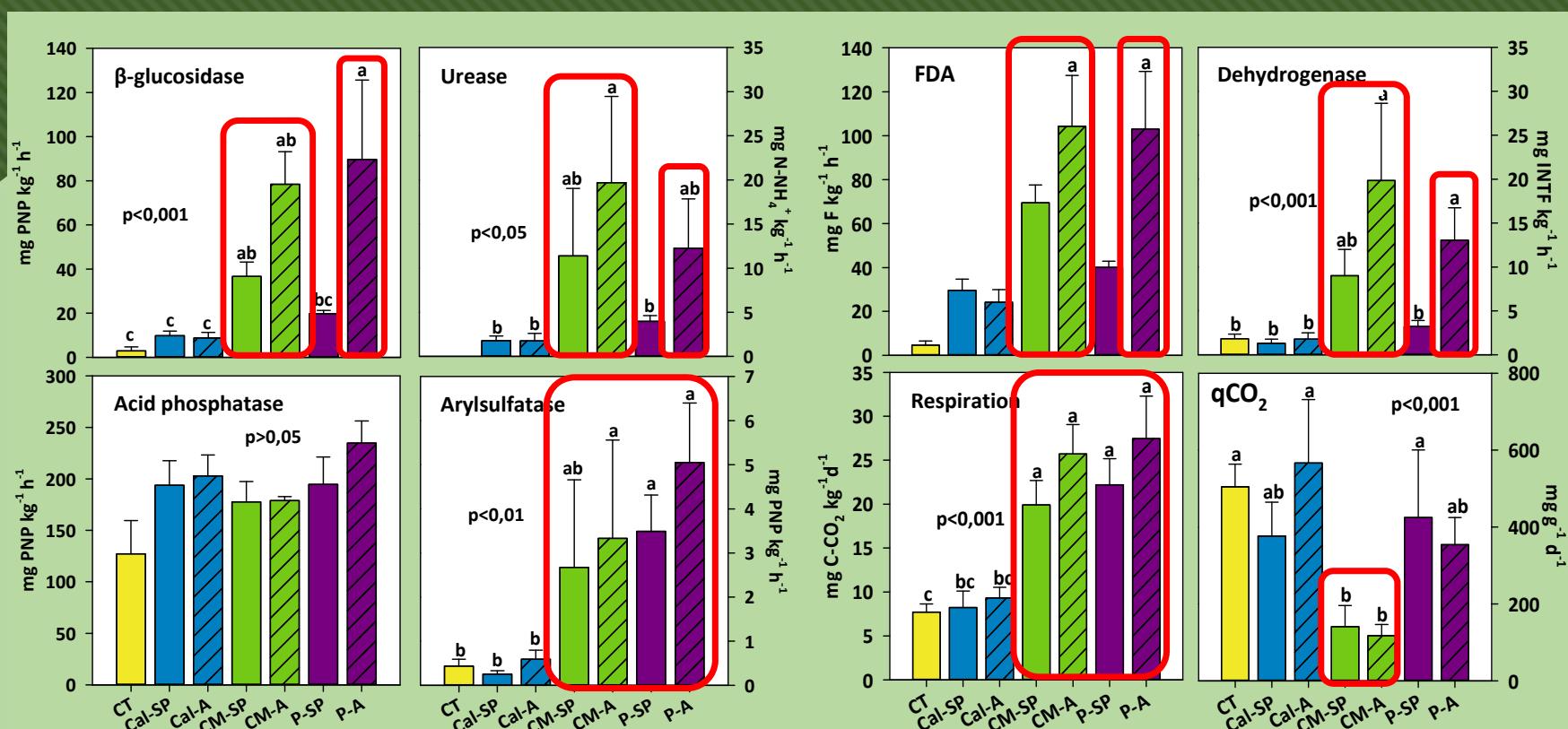
2 years after amendments addition



Case study 2 – Microbial indicators

Treatment	B_C mg kg ⁻¹	B_N mg kg ⁻¹
Cal-SP	24 c	0.3 c
Cal-A	20 c	0.2 c
CM-SP	167 a	10 a
CM-A	250 a	20 a
P-SP	29 bc	0.4 bc
P-A	91 ab	4.0 ab
CT	16 c	0.7 bc
T	***	**
PI	ns	ns
TxPI	***	***

Biomass



Functional activity: hydrolases (C, N & S cycle; catalytic potential (FDA); microbial community (dehydrogenase, respiration, qCO_2)

Case study 2 – Microbial indicators

CLPPs (community level physiological profiles): BIOLOG ECOPLATES



Average well color development (AWCD): overall functional activity of soil

Treatment	S	H'	AWCD
Cal-SP	6.0 ab	2.7 a	0.10 ab
Cal-A	7.0 ab	2.4 a	0.11 ab
CM-SP	14.8 a	3.6 a	0.38 a
CM-A	17.0 a	3.7 a	0.43 a
P-SP	8.0 ab	2.3 a	0.23 ab
P-A	12.3 a	3.2 a	0.23 ab
CT	1.2 b	0.4 b	0.01 b
T	***	***	
PI	ns	ns	
TxPI	**	**	

Functional diversity: S: species richness (number of substrates used); H': Shannon similarity index (uniformity in the distribution of the microbial community)

Case study 2 – Eco-toxicological tests

Plant growth test (direct) - *Zea mays* & *Lactuca sativa*



LC_{50} & EC_{50} : dose of contaminated soil (v/v) that provokes 50% emergence/growth inhibition, respectively. NT: non toxic

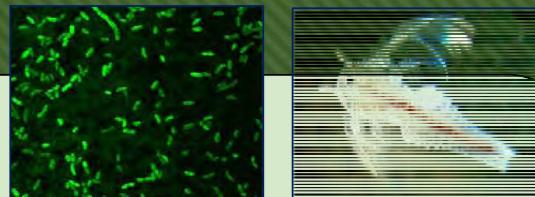
Treatment	<i>Z. mays</i>		<i>L. sativa</i>	
	LC_{50}	EC_{50}	LC_{50}	EC_{50}
Cal-SP	NT	85.75	58.19	20.02
Cal-A	NT	80.50	46.68	19.73
CM-SP	NT	NT	NT	18.31
CM-A	NT	NT	NT	NT
P-SP	NT	93.31	59.86	13.39
PCT-A	NT	91.70	NT	NT
CT	NT	70.85	36.68	4.95

Case study 2 – Eco-toxicological tests

EC_{50} (v/v)

Indirect assays:

- *Vibrio fischeri* (luminescence inhibition test)
- *Thamnocephalus platyurus* (mortality test)
- *Lepidium sativum* & *Lactuca sativa* (germination test)



Treatment	<i>V. fischeri</i>		<i>T. platyurus</i>
	t = 15 min	t = 30 min	
Cal-SP	62,52	57,97	78,83
Cal-A	60,76	59,77	73,53
CM-SP	62,55	61,15	93,82
CM-A	47,98	45,79	84,76
P-SP	54,01	49,51	82,28
P-A	62,29	60,86	78,78
CT	61,69	61,46	10,15

<i>L. sativum</i>			
Treatment	G (%)	R(%)	GI(%)
Cal-SP	87,8	51,8 d	45,4 c
Cal-A	84,7	51,9 d	43,9 c
CM-SP	91,8	64,0 c	58,7 b
CM-A	96,9	71,8 ab	69,6 a
P-SP	91,8	67,9 bc	62,3 ab
P-A	88,8	72,6 a	64,4 ab
CT	87,8	47,0 d	41,3 c
ANOVA	ns	***	***

<i>L. sativa</i>			
Treatment	G(%)	R(%)	GI(%)
Cal-SP	90	67,3 cd	60,5 bc
Cal-A	94	69,1 bcd	64,9 bc
CM-SP	99	92,9 abc	92 ab
CM-A	99	96,9 abc	95,9 a
P-SP	99	96,2 ab	95,2 a
P-A	98	105,8 a	103,6 a
CT	92	51,2 d	47,3 c
ANOVA	ns	**	**

Case study 2 – CONCLUSIONS

- ✓ Soil toxicity: high salinity, acidic pH and TEs solubility; amendments led to a reduction of their potential associated risks
- ✓ Supply of essential nutrients (pig slurry and compost) promoted a stable vegetation cover, that supposed an extra input of nutrients
- ✓ Both organic amendments in combination with *A. halimus* stimulated the soil microbial communities
- ✓ The combination of compost or pig slurry with *A. halimus*: effective strategy to improve soil health of TEs-contaminated soils under semi-arid conditions

Case study 3 - Restoration of a TEs contaminated agricultural soil



Case study 3 - Restoration of a TEs contaminated agricultural soil



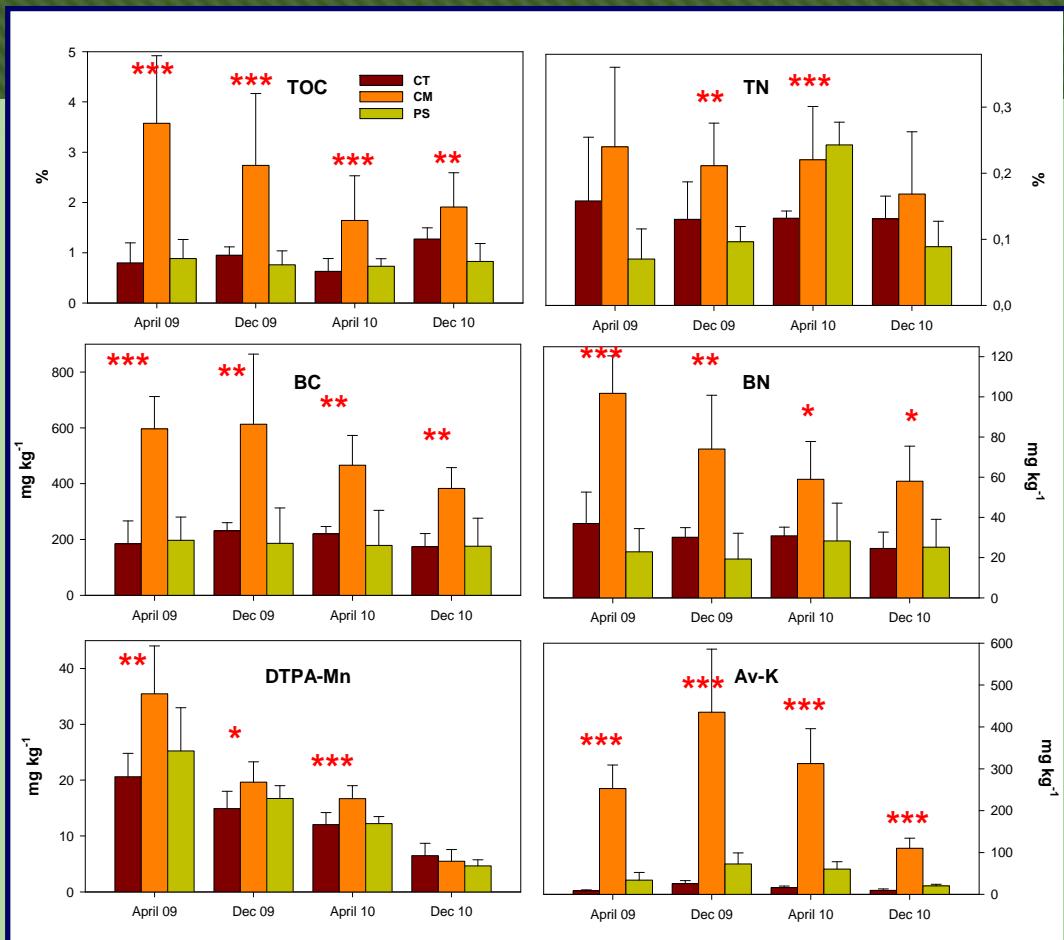
Case study 3 - Restoration of a TEs contaminated agricultural soil



Atriplex halimus L.

Bituminaria bituminosa L.

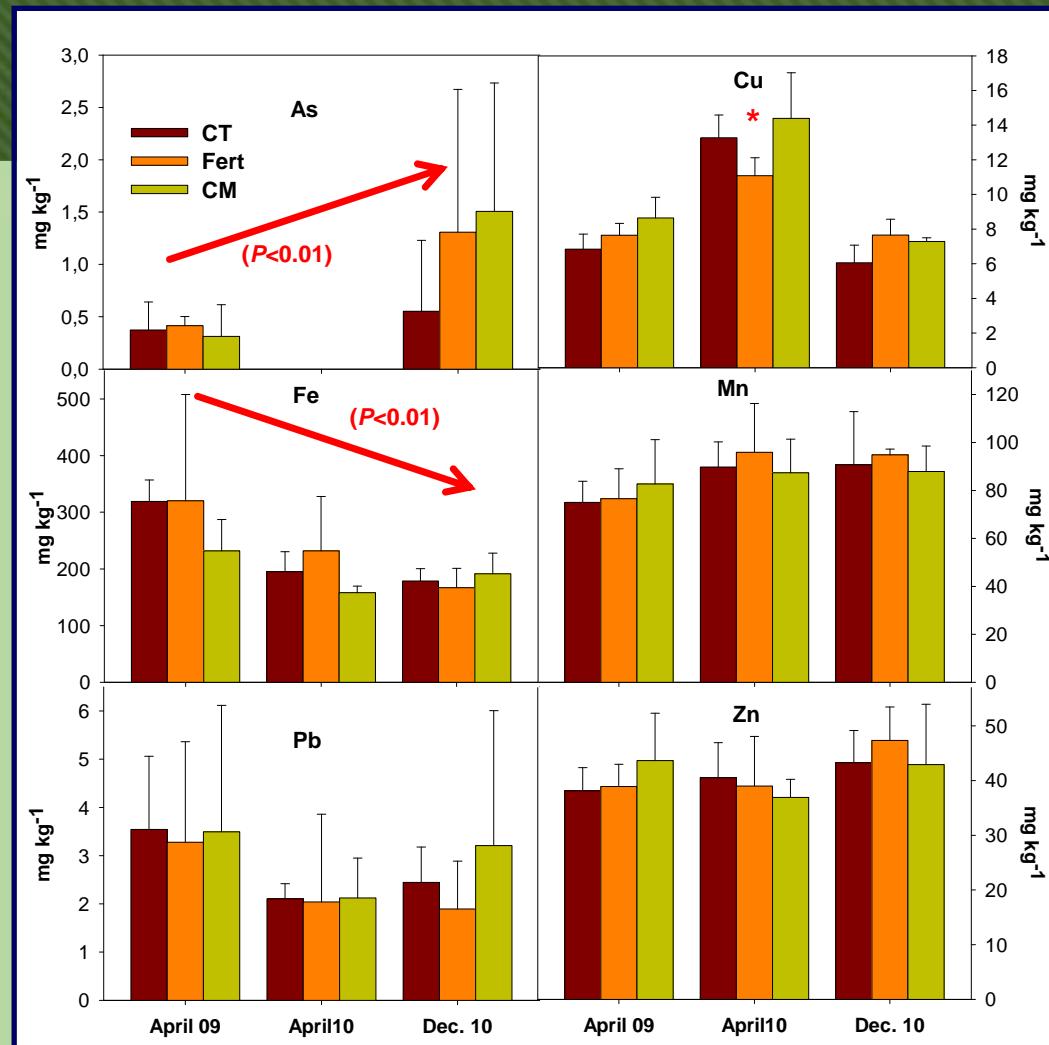
Case study 3 - SOILS: TREATMENT EFFECTS



	Pb	Cd	As	C_{OT}	K	P
B_C	-0,347*	-0,307*	-0,458***	0,694***	0,771***	0,658***
B_N	-0,322*	-0,303*	-0,451***	0,758***	0,679***	0,661***

Pearson's Coefficient (r). *: $p<0,05$; **: $p<0,01$; ***: $p<0,001$

Case study 3 - PLANTS (*B. bituminosa*)



Case study 3 - PLANTS (spontaneous vegetation)



Ecological indexes
(number of plant species and
individuals)

Case study 3 - PLANTS (spontaneous vegetation)

Familia	Especie
Apiaceae	<i>Eryngium campestre</i>
Asteraceae	<i>Atractylis cancellata</i>
	<i>Carduus bourgeanensis</i>
	<i>Carthamus lanatus</i>
	<i>Crepis vesicaria subsp haenseleri</i>
	<i>Dittrichia viscosa</i>
	<i>Leontodon taraxacoides</i>
	<i>Phagnalon saxatile</i>
	<i>Reichardia tingitana</i>
Boraginaceae	<i>Cynoglossum creticum</i>
	<i>Echium creticum subsp. coincyanum</i>
	<i>Echium sabulicolum</i>
Brassicaceae	<i>Lobularia maritima</i>
Chenopodiaceae	<i>Atriplex halimus</i>
Cistaceae	<i>Fumana laevis</i>
Convolvulaceae	<i>Convolvulus althaeoides</i>
Crassulaceae	<i>Sedum sediforme</i>
Fabaceae	<i>Bituminaria bituminosa</i>
	<i>Coronilla scorpioides</i>
	<i>Scorpiurus sulcatus</i>
Frankeniaceae	<i>Frankenia corymbosa</i>
Myrsinaceae	<i>Anagallis arvensis</i>
Lamiaceae	<i>Lavandula multifida</i>
	<i>Thymus hyemalis</i>
Poaceae	<i>Phalaris brachystachys</i>
	<i>Stipa capensis</i>
Rubiaceae	<i>Galium verrucosum</i>
	<i>Valantia hispida</i>
Xanthorrhoeaceae	<i>Asphodelus fistulosus</i>
TOTAL	29

- ✓ 20.1% woody, 79.3% herbaceous
- ✓ No amendment effect on species distribution & abundance
- ✓ Compost > plant cover %

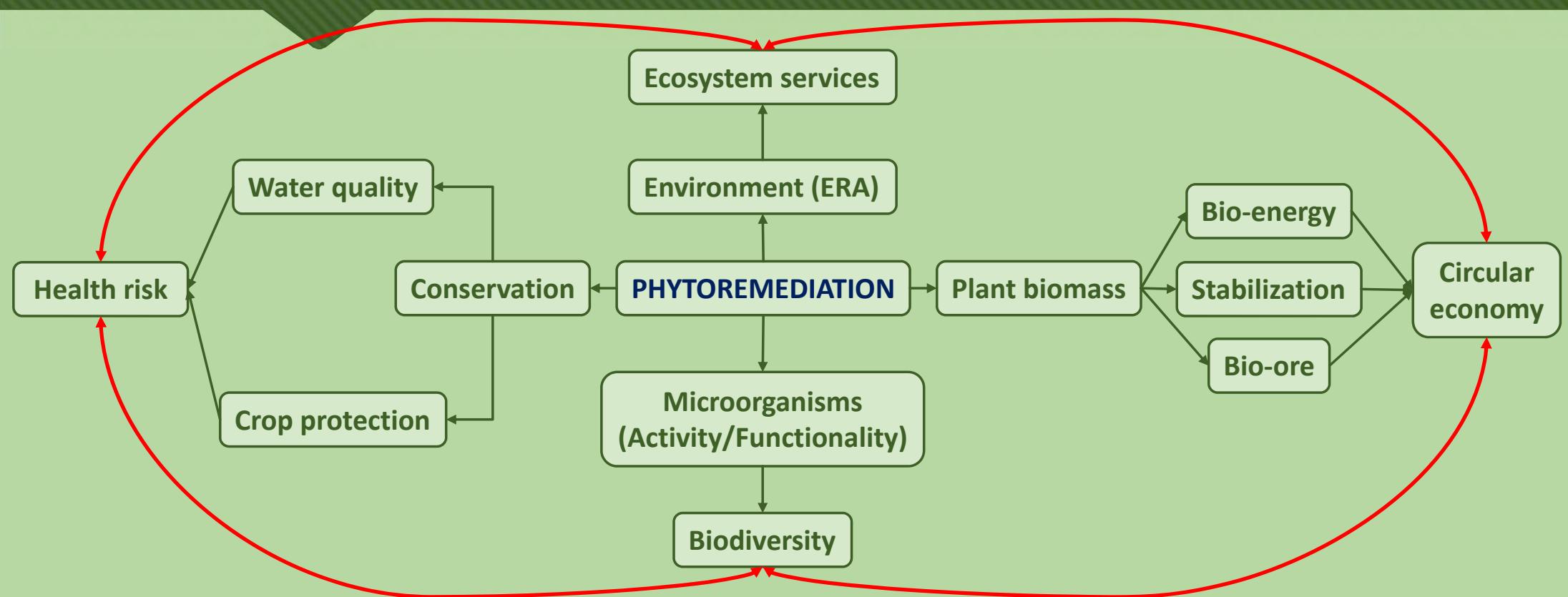


Pardo et al., 2014. Env. Sci. Poll. Res. 21, 1029-1038.

Case study 3 - CONCLUSIONS

- ✓ TEs present mostly in hardly-available forms; amendments application **did not alter extractability**
- ✓ Long-term supply of essential nutrients (compost): **development of the microbial community** and improved the plant growth (**greater vegetation cover**)
- ✓ Compost: **useful for phytostabilization** of moderately polluted soils, but combination with other species must be studied

Phytoremediation: Closing the loop



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School of Soil Biodiversity and Bioindication

*Biodiversity and bioindicators in monitoring
and management of contaminated soils*



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