GOLD

Bridging the gap between phytoremediation solutions on growing energy crops on contaminated lands and clean biofuel production

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WP1. Optimization of selected high-yielding lignocellulosic energy crops for phytoremediation purposes and biofuel production

5 polluted sites in Europe and 2 in Asia

- **Small-scale pot trials in a greenhouse**
- **Pilot-scale field trials**

Selected energy crops

Crop characteristics:

- ✓ high yielding, giving biomass suitable for bioenergy production
- ✓ relatively tolerant to HM&M, with ability to grow on metal-contaminated soils
- ✓ have relatively low agricultural requirements

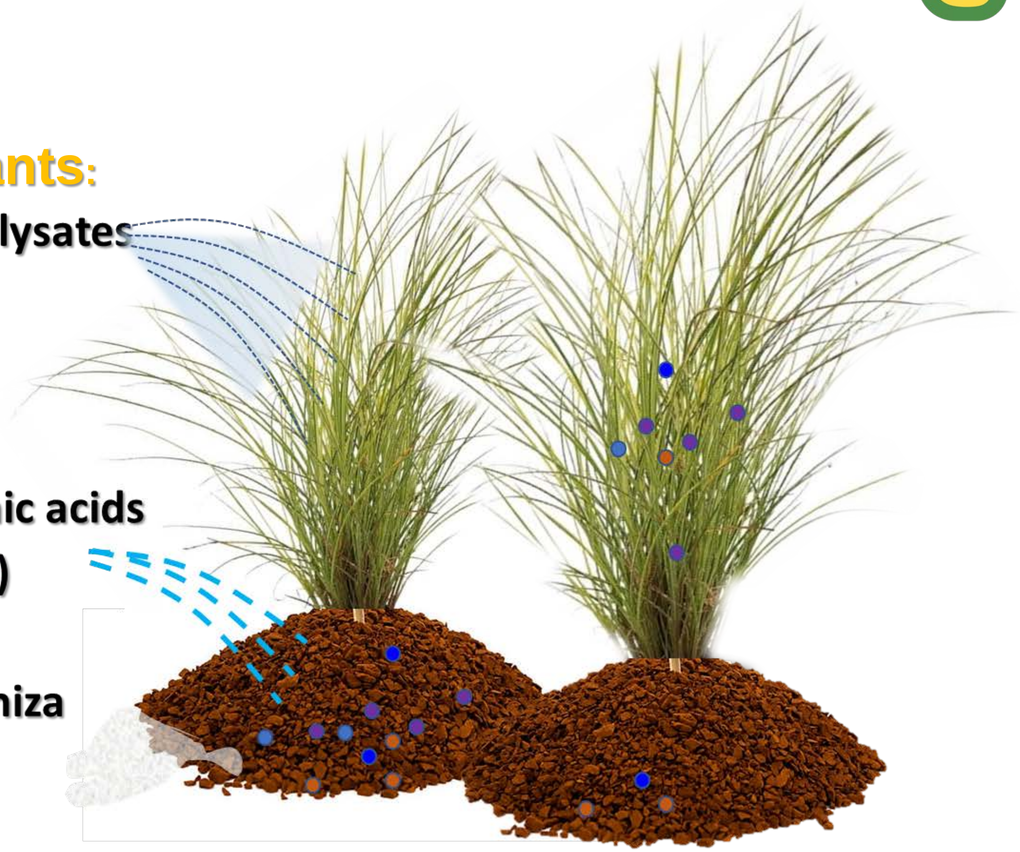
Treatments

Biostimulants:

protein hydrolysates (B1)

fulvic/humic acids (B2)

mycorrhiza (M)

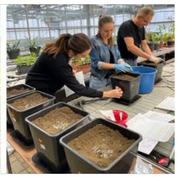


Control
B1
B2
M
B1xM
B2xM

1st step: Pot trials

UMCS, Poland





AUA, Greece

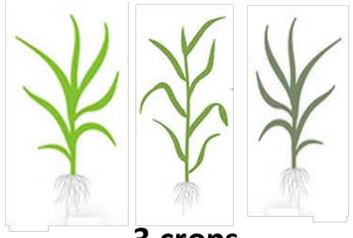


YNCREA, France



HUNAU, China

Soil has been collected from the experimental fields (physico-chemical characteristics and pollutant content)

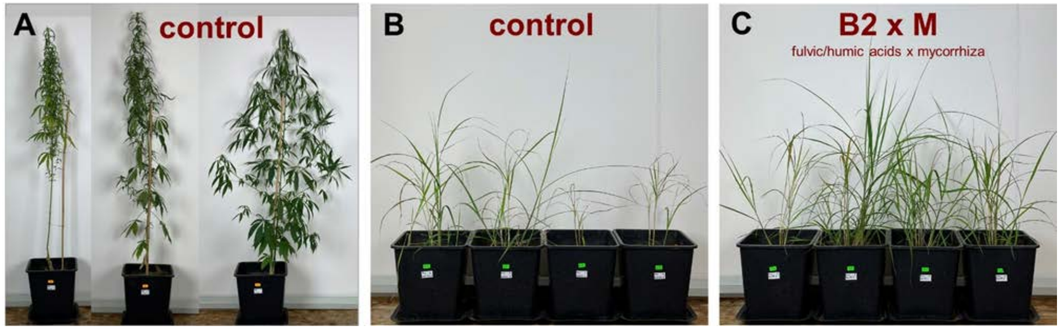


3 crops
x 6 treatments
x 4 repetitions

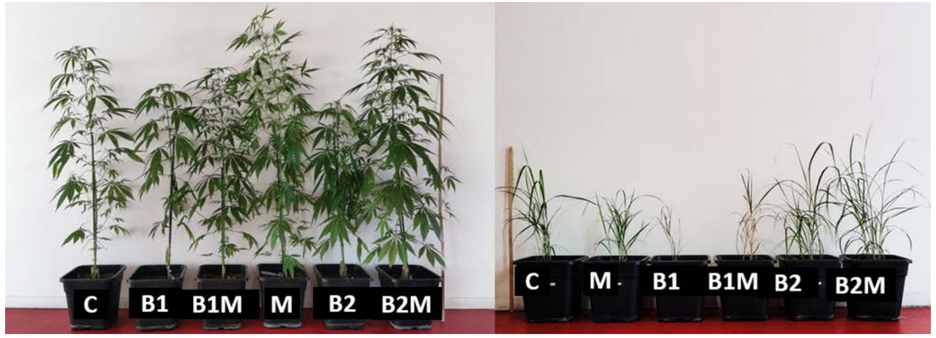
72 pots + 4 pots without plants = 76 pots

Pot size: 26x26 cm =12 lit = approx. **12 kg of soil per pot**. Thus, **912 kg of soil** from each field was needed to fill the 76 pots

UMCS

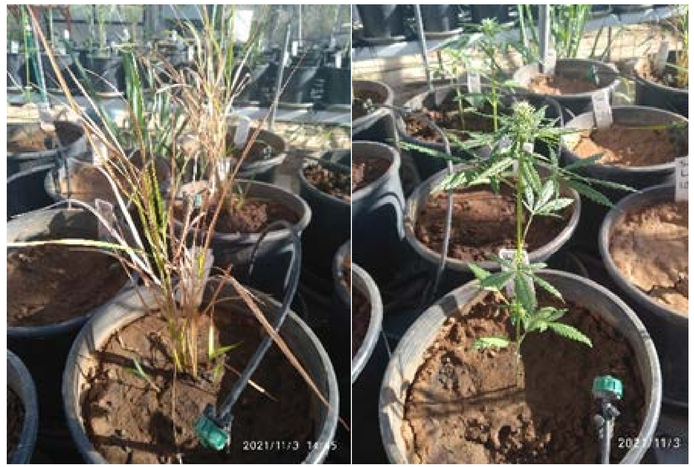


JUNIA



Final treatments to be applied at the fields

AUA

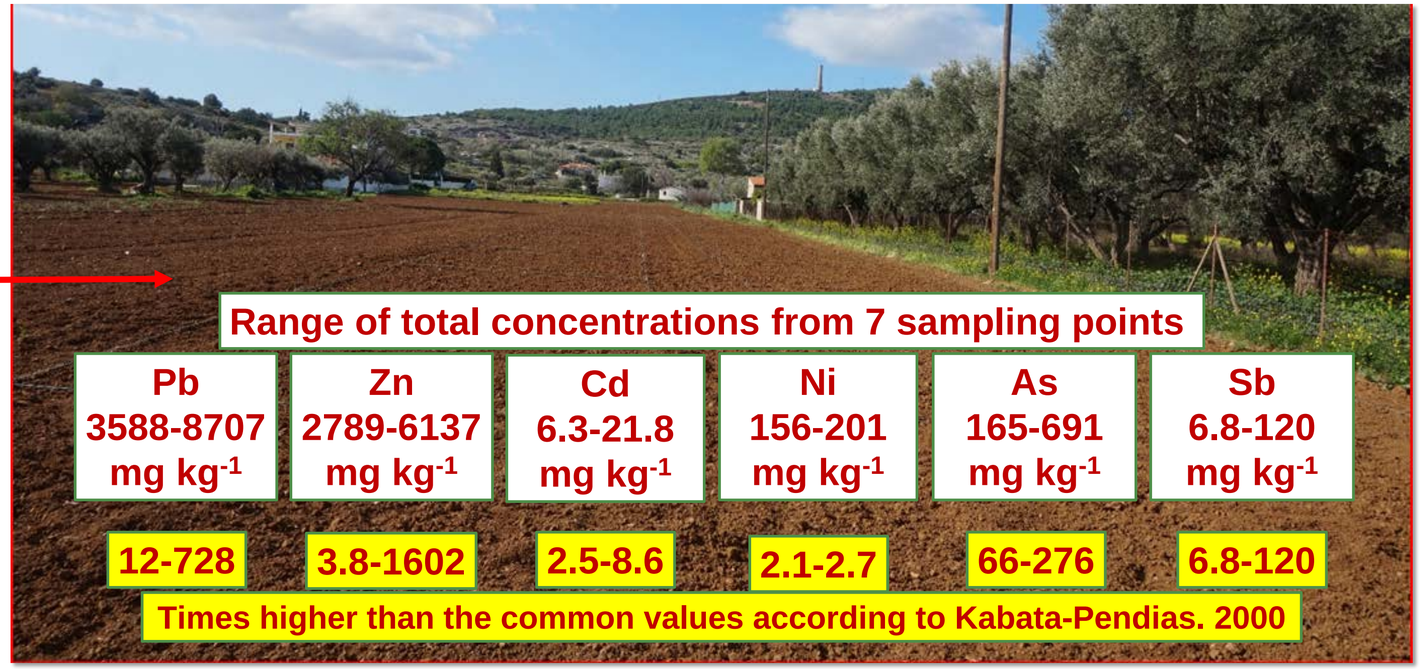


CRES

Partner	Plant species	Treatments selected for field trials					
		B1	B2	M	B1x M	B2xM	Control
UMCS, Poland	miscanthus		X			X	X
	industrial hemp		X			X	X
	sorghum		X			X	X
AUA, Greece	miscanthus			X		X	X
	industrial hemp			X		X	X
	sorghum			X		X	X
CRES, Greece	miscanthus				X	X	X
	sorghum				X	X	X
	switchgrass				X	X	X
UNIBO, Italy	miscanthus		X			X	X
	industrial hemp	X				X	X
	sorghum				X	X	X
YNCREA, France	miscanthus		X			X	X
	industrial hemp		X			X	X
	sorghum		X			X	X
IBFC, China	industrial hemp → **kenaf**	X				X	X
	sorghum			X		X	X
HUNAU, China	miscanthus				X	X	X
	switchgrass				X	X	X

Experimental fields in GREECE (two sites)

1st site: in Lavreotiki peninsula (AUA)



Range of total concentrations from 7 sampling points

Pb	Zn	Cd	Ni	As	Sb
3588-8707 mg kg^{-1}	2789-6137 mg kg^{-1}	6.3-21.8 mg kg^{-1}	156-201 mg kg^{-1}	165-691 mg kg^{-1}	6.8-120 mg kg^{-1}
12-728	3.8-1602	2.5-8.6	2.1-2.7	66-276	6.8-120

Times higher than the common values according to Kabata-Pendias. 2000

Long term multi-contaminated site.
Ancient (3000-200 B.C.) and more recent (1864-1982 A.D.) mining and metallurgical activities resulted in a heavy soil contamination of the area, mainly with Pb, Zn, Cd, Ni, As, Sb.

Hemp

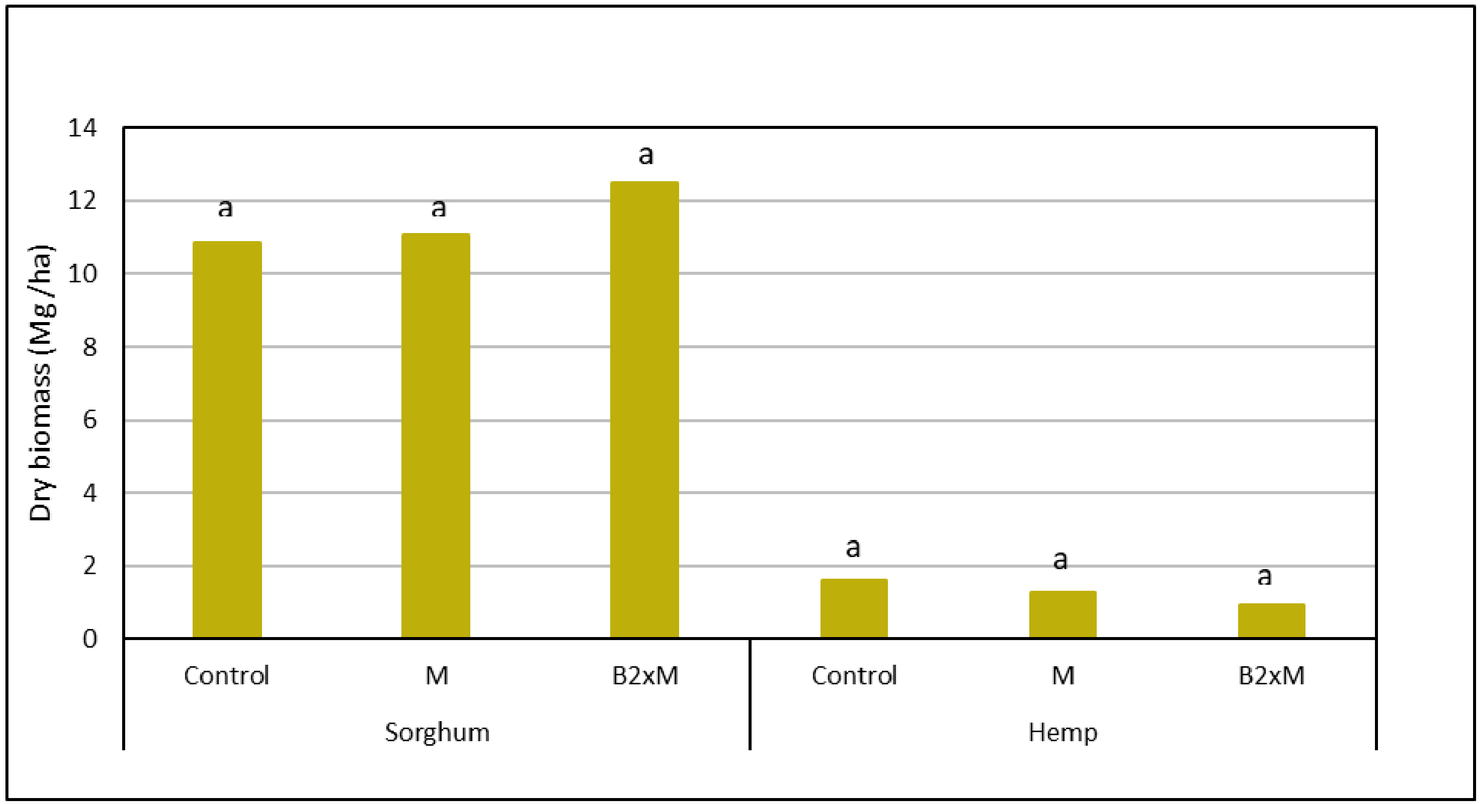
27 September 2022

Sorghum

Miscanthus

-Plant growth has stopped
-Stems remained thin
-Life cycle was shorten
(panicles in 50 cm height)

Dry biomass of sorghum and hemp from the heavily contaminated site of Lavrion (AUA field)



Treatments:

M=mycorrhiza

B2XM= fulvic/humic acids+ mycorrhiza

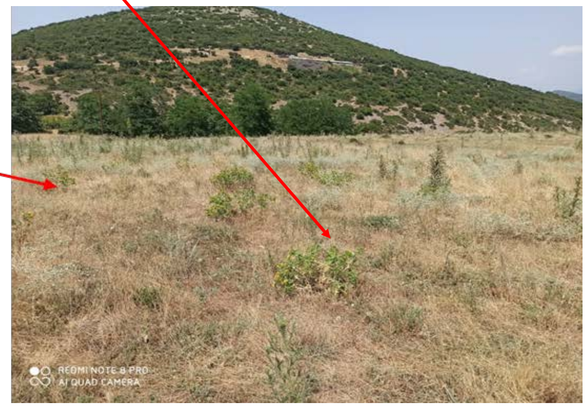
AUA field: HM&M concentrations in plant tissues-initial measurements

- ✓ Thirty elements were measured by our Polish partner UMCS
- ✓ All were above the detection limits of the ICP-MS
- ✓ The results of the below table are per treatment.

TREATMENTS	Concentrations in plant tissues collected from the AUA field (mg/kg)					
	Pb	**Zn**	**Ni**	**Cd**	**As**	**Sb**
Hemp- Control	32.36	60.68	0.71	1.81	1.70	0.07
Hemp-Mycorrhiza	35.57	63.11	1.06	1.47	1.67	0.07
Hemp Mycorrhiza+Lonite	42.27	144.80	1.34	1.48	1.74	0.18
Hemp Myc+Siapton+ Lonite	51.64	124.29	3.38	1.71	1.64	0.18
Sorghum- Control	47.23	354.43	0.85	11.98	0.34	0.04
Sorghum - Mycorrhiza	78.22	528.98	1.13	21.57	0.86	0.08
Sorghum- Mycorrhiza+Lonite	94.73	341.56	0.72	28.04	0.88	0.13
Usual/normal values (Kabadas-Pendias 2001)	0.2-20	1-400	2.02-5	0.2-2.4	0.02-7	0.0001-0.2

2nd GREEK site: in Kozani (CRES & METE)

Site of CRES field trials



	Sampling points								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
Zn	52.4	45.7	54.2	45.8	52.4	43.0	44.7	46.0	47.8
Cu	15.7	13.5	16.0	12.7	15.1	12.8	13.6	13.5	13.8
Pb	7.9	6.1	7.1	7.1	7.3	6.3	6.6	6.6	5.9
Ni	1103	993	1386	829	708	721	1021	997	1115
Cd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
As	1.16	1.14	1.20	1.10	1.19	1.07	1.20	1.20	1.25
Sb	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
pH	7.9	7.9	8.0	7.8	7.8	7.8	7.9	7.8	7.9

Site located nearby a lignite mining area polluted with ash containing several contaminants.
Main pollution by nickel.

22 June 2022

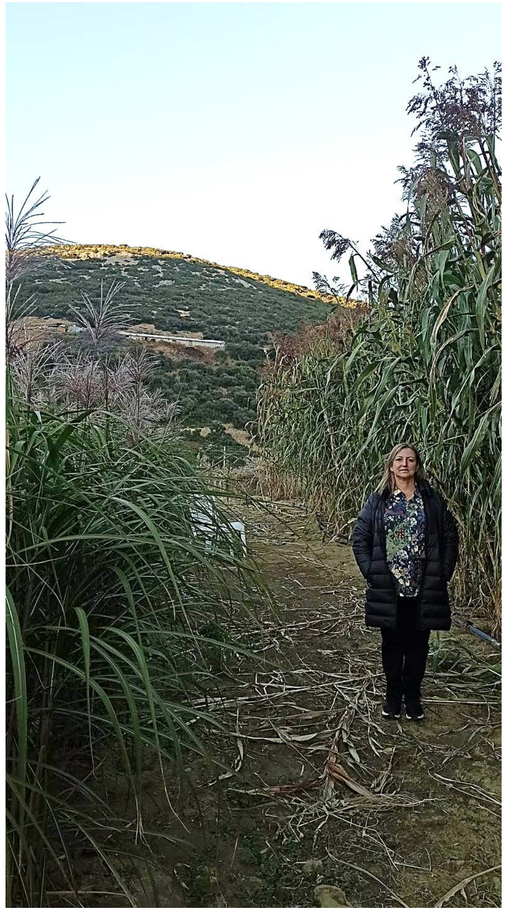
Sorghum

Miscanthus

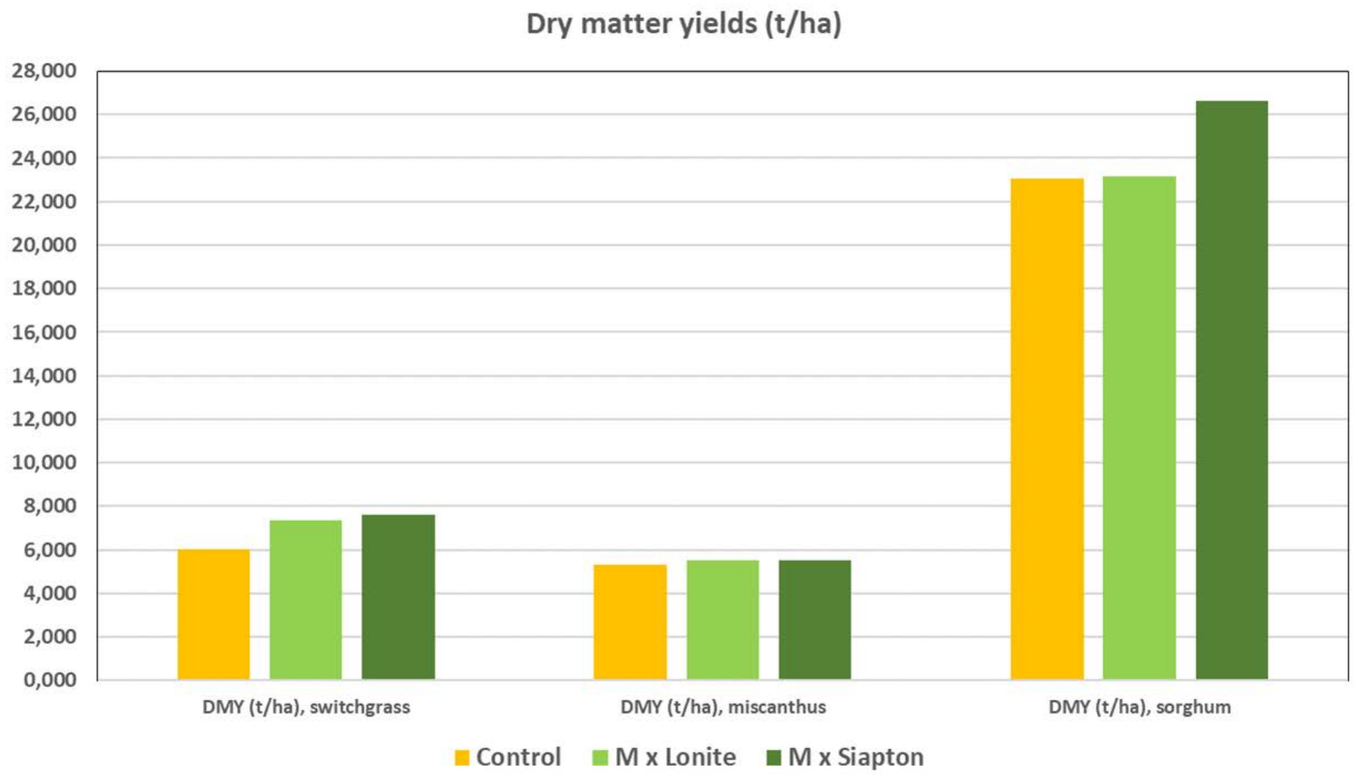
Switchgrass

22 October 2022

Sorghum Miscanthus Switchgrass

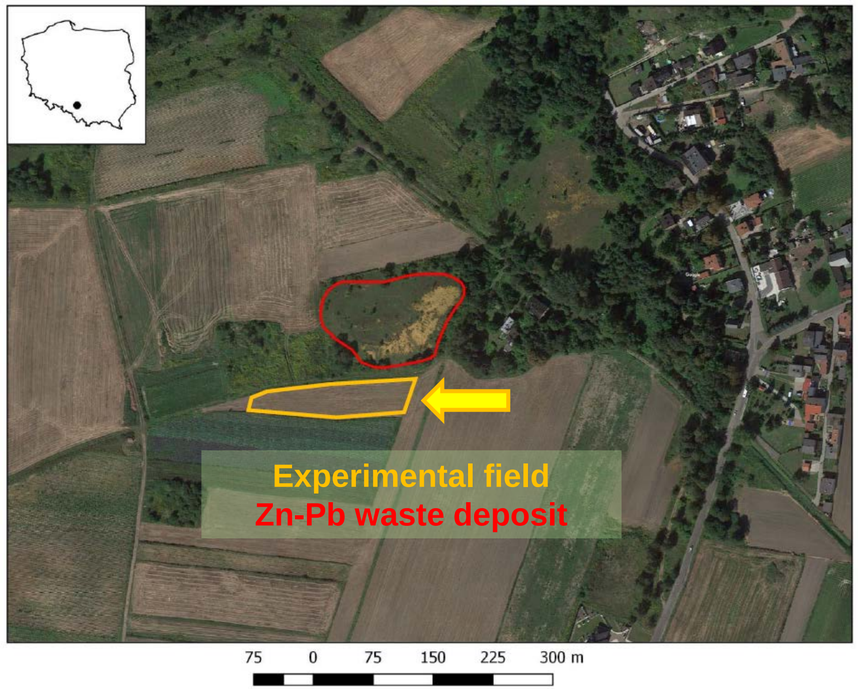


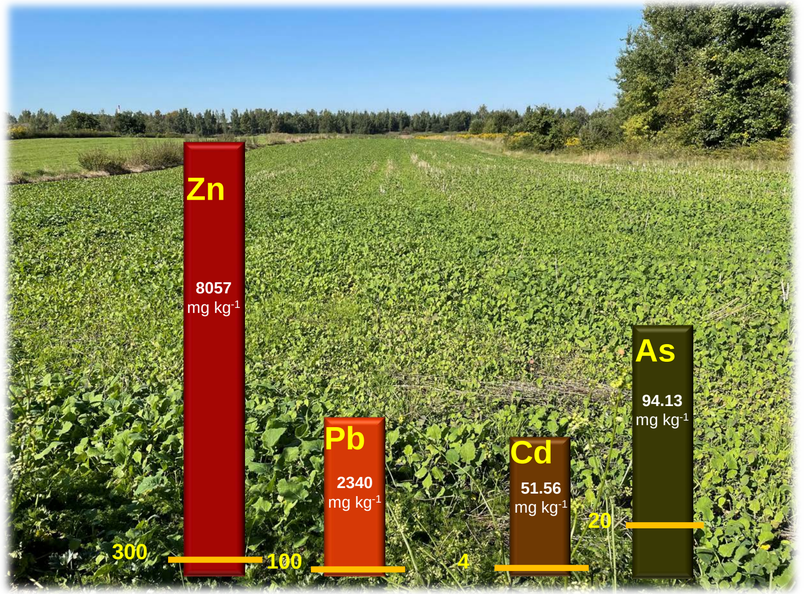
Dry biomass of switchgrass. miscanthus and sorghum from the contaminated site of Kozani (CRES field)



Experimental field in POLAND (UMCS)

Region of Upper Silesia (UMCS)





A long-term multi-metal contaminated site due to mining and metallurgical activities (dating back to 19 century), neighboring from the north with an old metalliferous waste dump.

Exceess concentrations of zinc, lead, cadmium, arsenic.

1) Control
2) B2 (humic/fulvic acids)
3) B2 x M (humic/fulvic ac. x myc.)

Hemp

C < B2xM < B2*

42* t / ha (FW), 3.1 m high

~8 t / ha (DW)

HM concentrations?

Sorghum

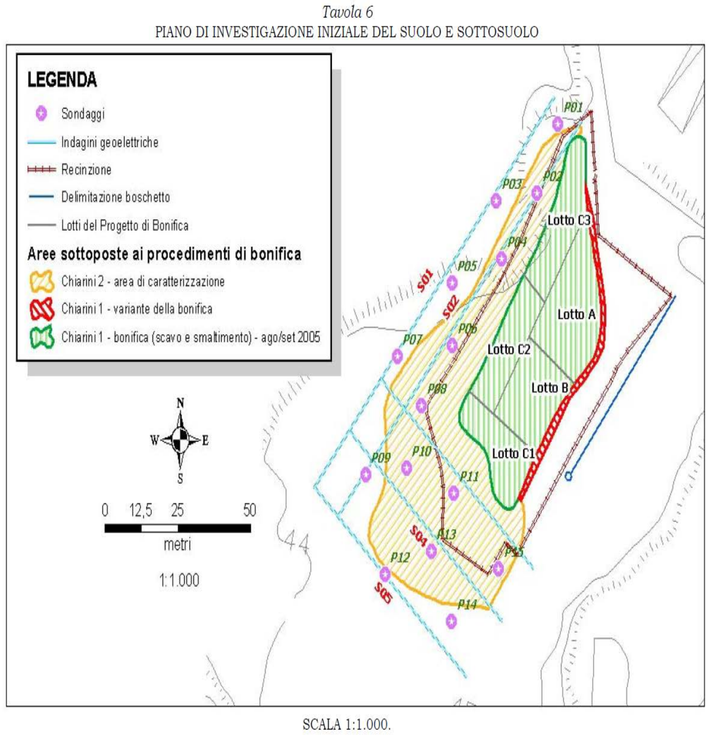
C < B2xM < B2*

115* t / ha (FW), 2.9 m high

~20 t / ha (DW)

Experimental field in ITALY (UNIBO)

Site CHIARINI 2, surroundings of Bologna city



Parameter	Result	U	U.M.	Legal Threshold
Lead	159	± 32		100
Copper	137	± 27		120
Nickel	209	± 42	mg kg^{-1} DM	120
Zinc	455	± 91		150
Tin	8.8	± 1.8		1

The site was part of an illegal dumpfill (Chiarini 1).
Main contaminants are Pb, Cu, Ni, Zn, and Sn in soil concentrations above the threshold established by Italian law.
Contamination of organic substances, PCBs, was detected. The complete characterization is still in progress.

UNIBO CROPS AND TREATMENTS

For each of the 3 crops tested in pots 3 treatments were selected to be applied in field

Sorghum vulgare: M*B1; M*B2; C

Cannabis sativa: B1; M*B2; C

Miscanthus x giganteus: B2; M*B2; C

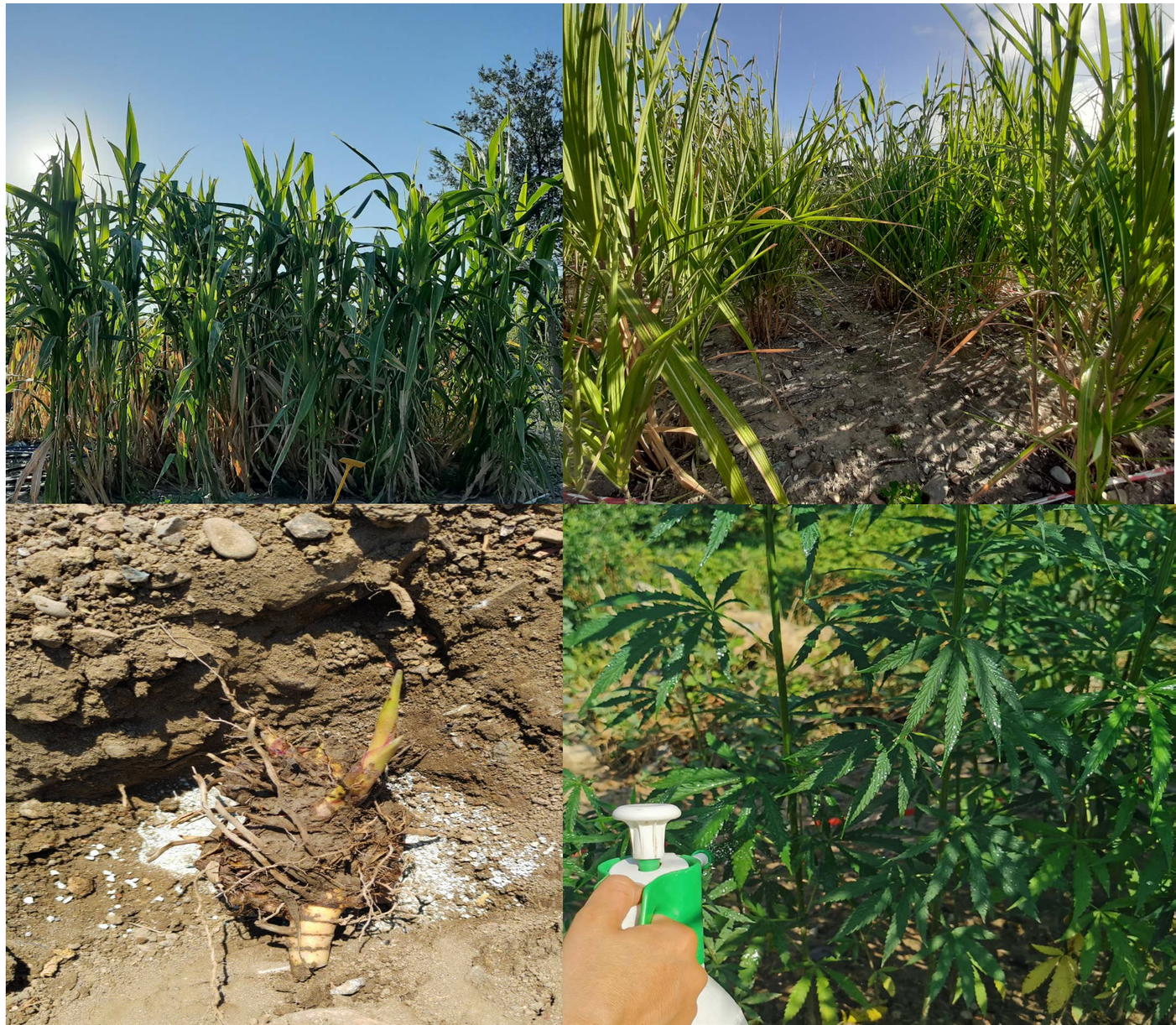
B1: foliar biostimulant

B2: radical biostimulant

M*B1: mycorrhiza with foliar biostimulant

M*B2: mycorrhiza with radical biostimulants

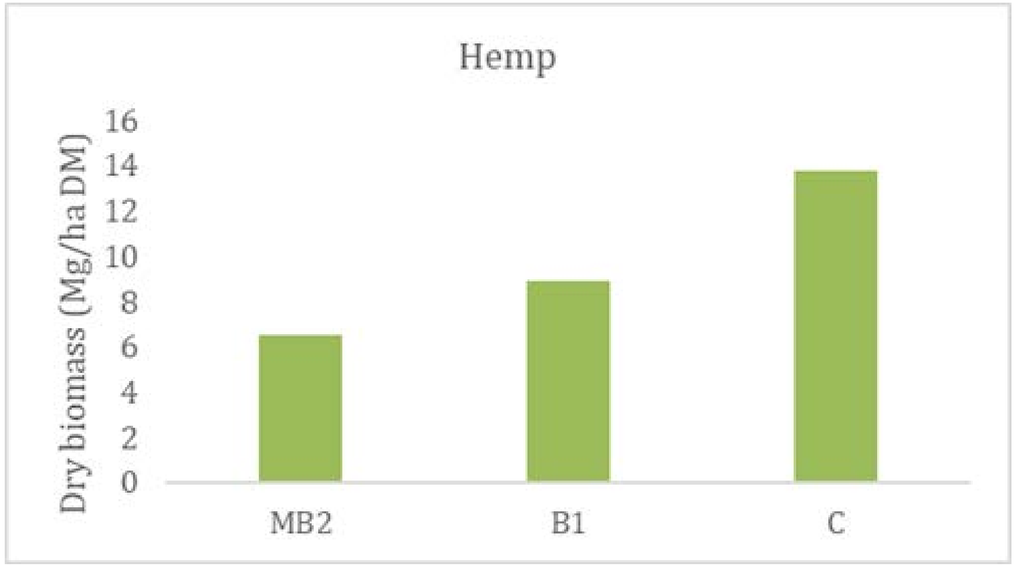
C: untreated control

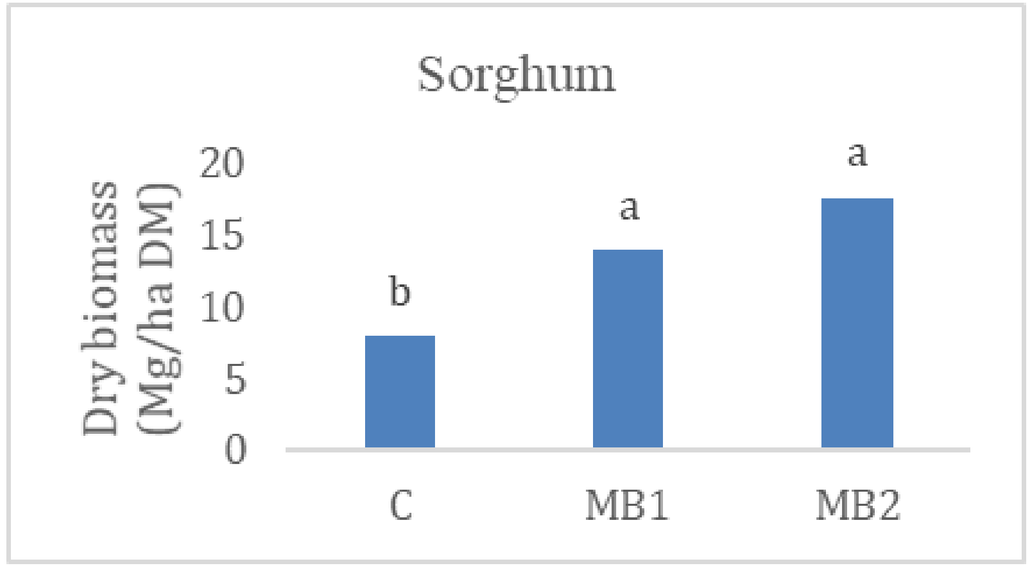


Hemp was harvested in August 2022 and sorghum in October 2022.

Miscanthus was harvested in January 2023 in order to allow nutrients reallocation in rhizomes.

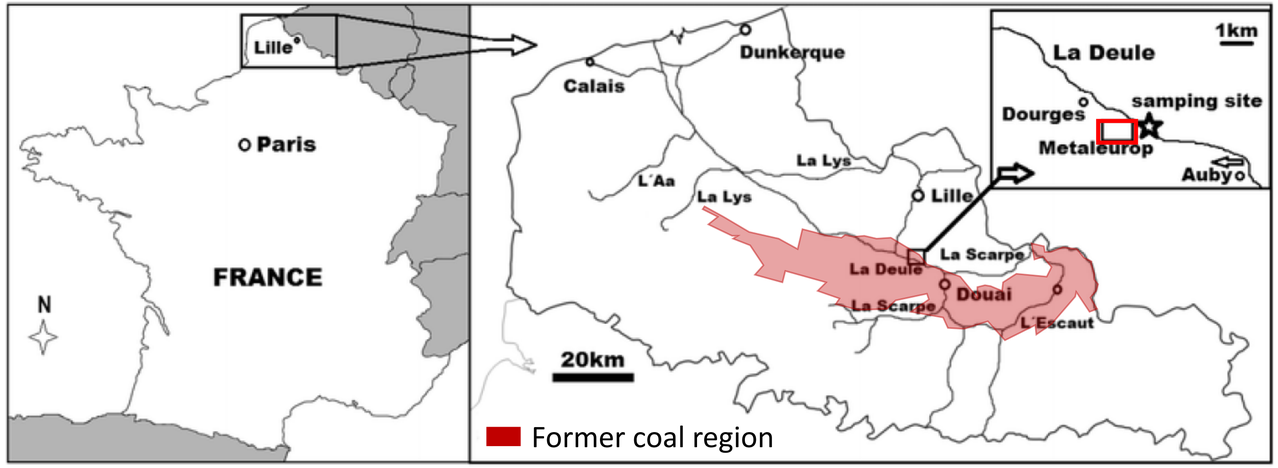
Inorganic and organic contamination analyses are in progress…

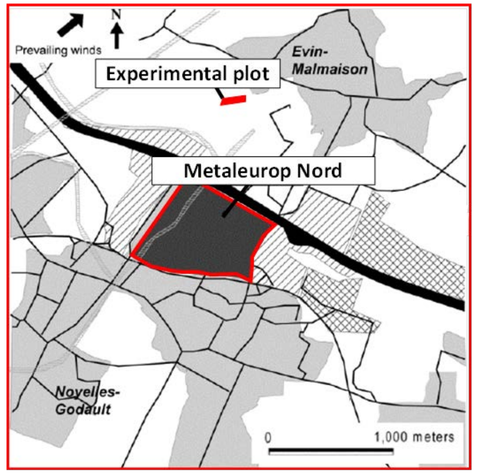


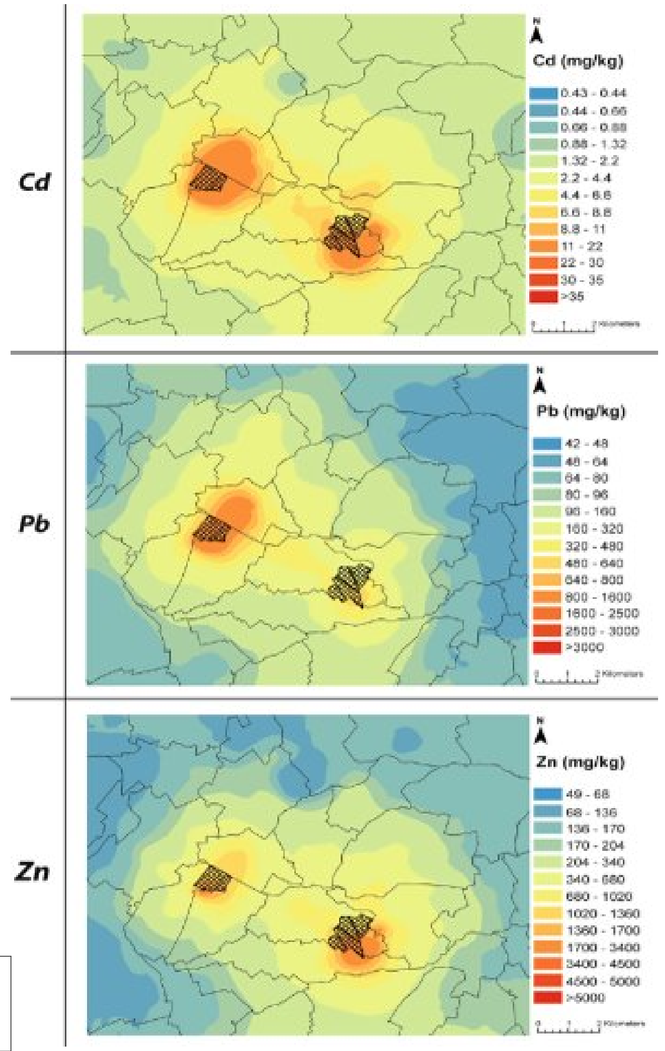


Experimental field in FRANCHE (JUNIA)

Site MetalEurop Nord in Noyelle-Godault

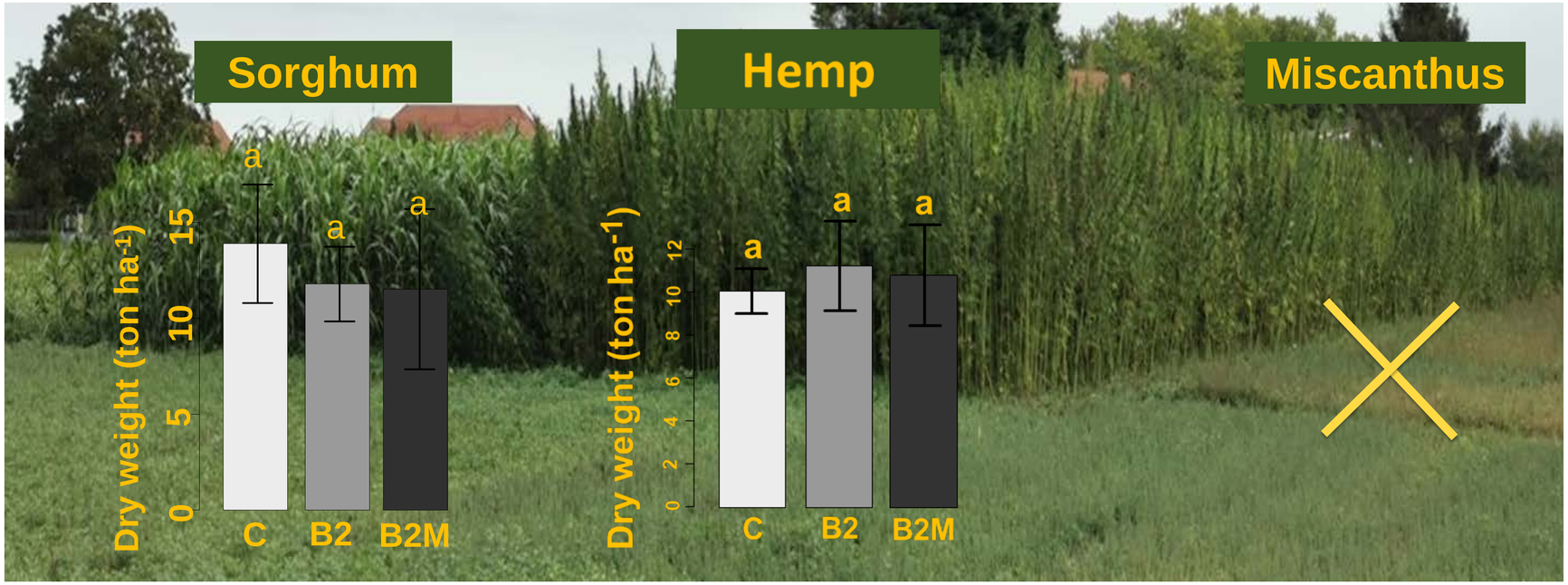


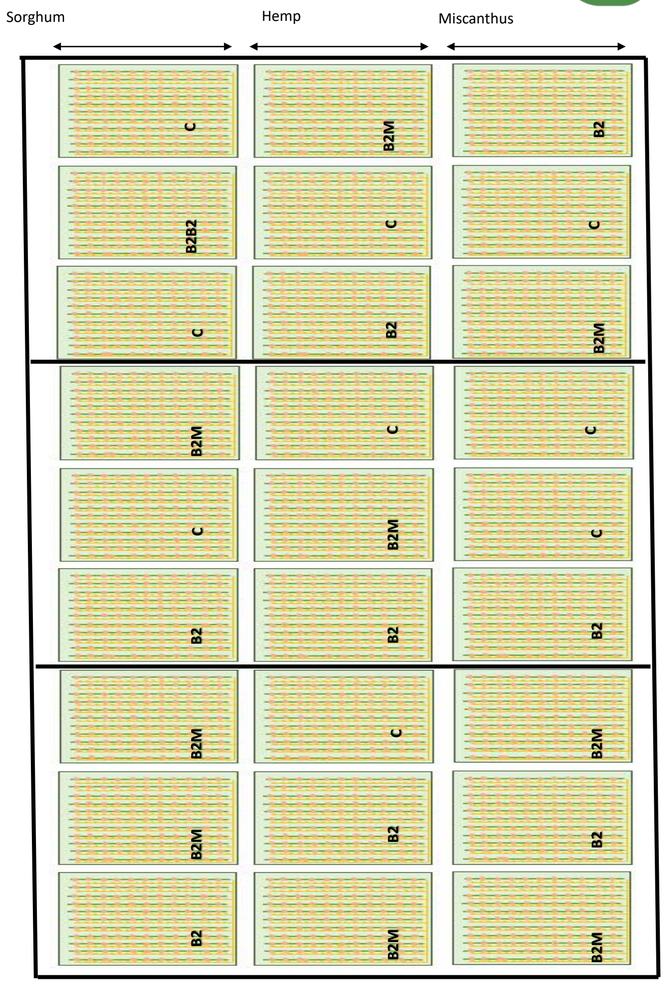




Experimental plot	**Tot Cd** (mg kg^{-1})	**Tot Pb** (mg kg^{-1})	**Tot Zn** (mg kg^{-1})	**pH**	**$CaCO_3$** (g Kg^{-1})	**Clay** (%)	**Silt** (%)	**Sand** (%)
	11 ± 2	536 ± 70	955 ± 151	7.9 ± 0.2	4.2	19	56	25
Control plot	0.3 ± 0.1	37 ± 1	54 ± 3	6.4	0.4	20.8	69.5	9.7

Pilot field trials – France – shoot DW yield





Hemp and sorghum before harvest

- No difference for shoot DW yield between treatments
- High shoot DW yield of sorghum and hemp; Drought impacted miscanthus production

Pilot field trials – France – Metal concentrations in shoots

	Sorghum (mg kg^{-1})			Hemp (mg kg^{-1})		
Treatment	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**
C	11.9±3.2a	13.5±1.8a	160±6a	0.8±0.1a	31.1±5.6a	211±18a
B2	13.0±3.4a	10.5±2.6a	166±21a	0.9±0.1a	29.4±5.6a	226±20a
B2xM	12.2±4.5a	11.7±2.3a	164±15a	0.8±0.1a	22.8±4.9a	194±24a

- No difference between treatments for metal concentrations in shoots and metal uptake
- Metal concentrations in shoots exceed the common concentration in shoots

Best yield per crop and partner

	Hemp		Sorghum		Switchgrass	
	Best Yield (DM t/ha)	Treatment	Best Yield (DM t/ha)	Treatment	Best Yield (DM t/ha)	Treatment
AUA	1.6	Control	12.5	B2xM		
CRES	-		26.6	B1xM	7.6	B1xM
UMCS	8	B2	20	B2		
UNIBO	13.8	C	17.63	B2xM		
JUNIA	11.2	B2	13.9	C		

GOLD

Thank you!

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ΚΑΠΕ CRES

Partners:

UMCS

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