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Gentle remediation of contaminated sites

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Soil contamination regarding **toxic metals and persistent organic pollutans** is a big concern around the world

! roughly million soil contaminated sites in European Union **!**

Phytoremediation – use of plants - soft remediation

- **Solar-driven** – Carbon sequestring technology
- Soil fertility - improvement
- Leaching - minimalization
- Erosion and dust transport - protection
- Ecologically friendly
- **Good community acceptance**

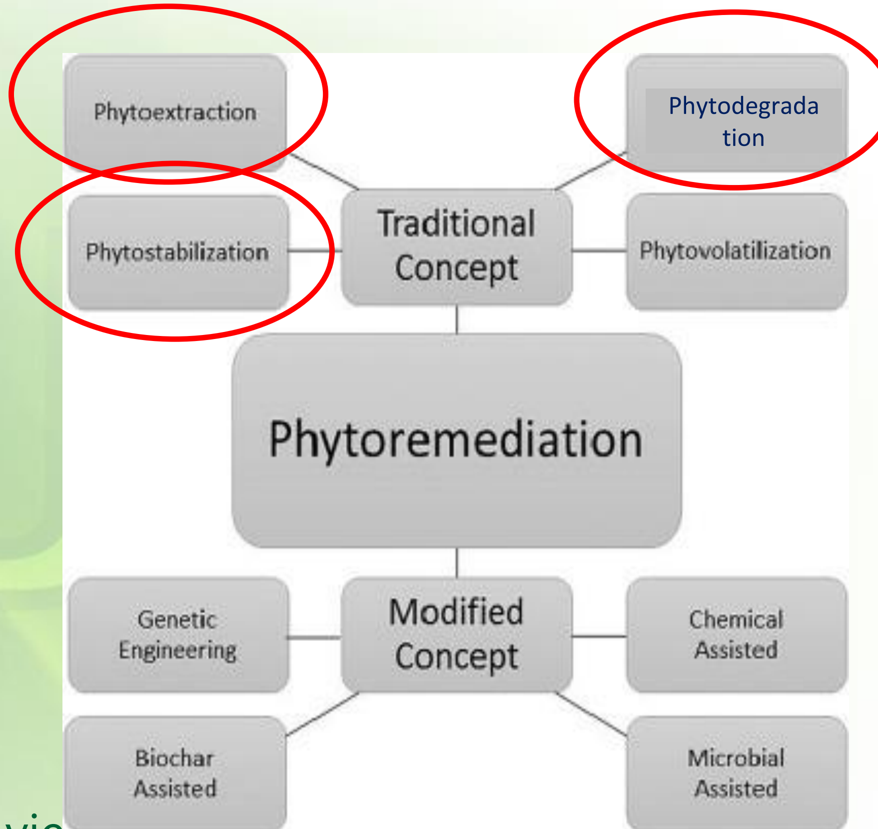
Bioremediation – use of microorgamisms - soft remediation

- Targeted application of microorganisms
- Degradation under thermophilic and mesophilic conditions

Phytoremediation

Phytostabilization – immobilization
sufficient growth is the most important

Amendment application
- limitation of metal mobility
- regulation of plant growth
  - Lime
  - Phosphates
  - Metal oxides
  - Composts and vermicomposts
  - Biomass ash
  - Biochar



Sarwar et al., 2017

Phytoextraction – removal
high metal accumulation and high biomass yield

Amendment application
- increase of metal mobility and uptake
- maximization of plant growth

- Chelating agents, Organic amendments, Acid fertilizers, Desorbing

Phytoremediation

Phytodegradation – decay
root activity and transformation of compounds

Bioavailabity of targeted compounds
Rhizosphere activity of roots
Degradation ability of above ground plants

Phytomanagement

Plant choice - hyperaccumulating /accumulating /excluding plants,
- annual x perennial,
- perennial – period of harvest, length of cultivation

Harvest - total x above ground

It is time to develop "**sustainable phytoremediation**"

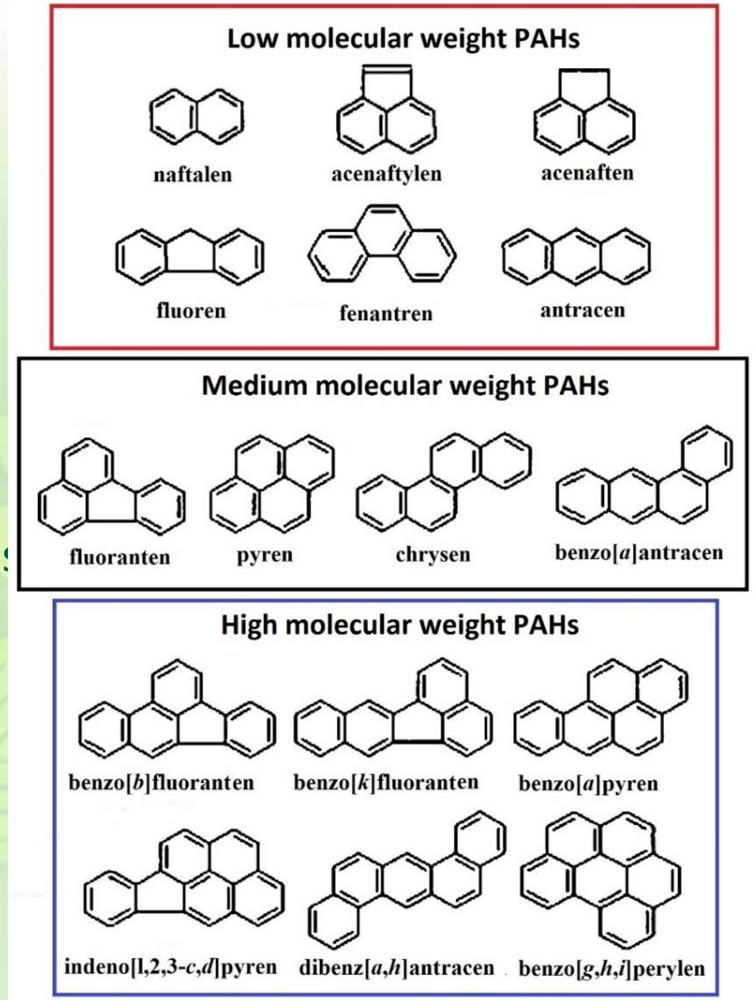
the promising option - energy crops.

Bioremediation

Bioaugmentation
targeted application of microorganisms

a) bioremediation using bacteria
- aerobic bacteria (*Pseudomonas* sp.)
- production of intracelular enzymes

b) bioremediation using fungi
- ligninolytic fungi (*Pleurotus* sp, *Irpex* s
- production of extracellular enzymes



[1]Anyakora et al. (2005)

Composting
microbial degradation of contaminants
under thermophilic and mesophilic conditions

Vermicomposting
„composting“ using earthworms
microbial degradation of contaminants under mesophilic conditions
earthworms additional support of degradation

Major objectives

Phytostabilization

The immobilization of metals on higly contaminated site

Role of lime

Role of biochar

Plant growth

Phytoextraction

The uptake of metals on medium contaminated soils

Pot experiment

Field experiment

- fertilization via sewage sludge
- period of harvest
- harvest in autumn or without

Fast growing trees with economical potential

willows

poplars

Metals removal by plants

Major objectives

Phytodegradation

The decay of polycyclic hydrocarbons (PAH) on spike and aged contaminated soil

Role of maize
Role of fungi

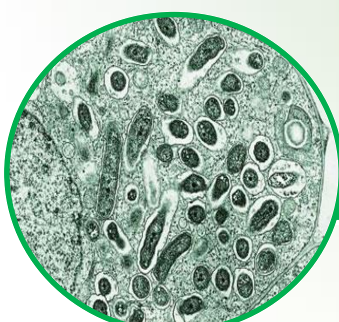
Plant – fungi interactions

Biodegradation

The degradation of polycyclic hydrocarbons (PAH) with the aid of organic amendments

Importance of composting and vermicomposting
Bioaugmentation of soils
Additional effect of plants

High biological activity



Bradyrhizobium sp.

Amanita muscaria

Phytostabilization - amendments

Extremely contaminated soil (Cd: 40 mg kg^{-1}; Zn: 5623 mg kg^{-1} ; Pb: 3706 mg kg^{-1})

Pot experiment- lime application

4 years experiment

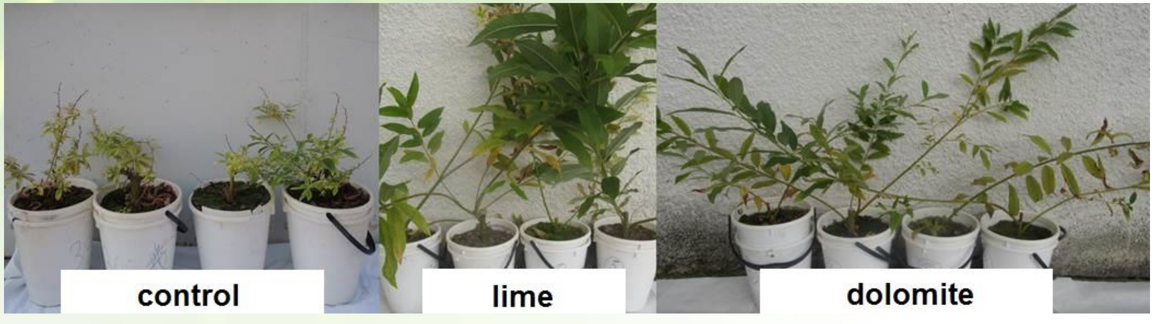
quick lime CaO or dolomite application $CaMg(CO_3)_2$

Mineral fertilization (0.1 g N; 0.16 g P; 0.4 g K per 1 kg)

Soil	Treatment	Growing season										
		1st-4th	Yield only 4th season									
		Total yield	Total yield	Non-harvestable: Coarse root	Non-harvestable: Fine root	Non-harvestable: Stump	Harvestable: Twig	Harvestable: Leaf	Harvest/ non-harvest	pH	Cd $CaCl_2$	Zn $CaCl_2$
Litavka	Control	89	32	3	3	21	1	3	0.13	5.8	3.21	173
	Lime 1	217	104	12	19	36	20	13	0.48	7.3	0.43	7.5
	Lime 2	229	126	14	21	46	32	15	0.67	7.7	0.15	4.2
	Dolomite 1	52	32	2	2	13	5.5	6	0.55	6.8	1.58	57
	Dolomite 2	121	60	12	23.5	23	8	9	0.31	6.7	1.42	45

Treatment abbreviations:
C – control soil without any additive
L1 – quick lime in dose 7.3 g kg^{-1}
L2 – quick lime in dose 21.9 g kg^{-1}
D1 – dolomite in dose 21.6 g kg^{-1}
D2 – dolomite in dose 68.1 g kg^{-1}

Litavka soil

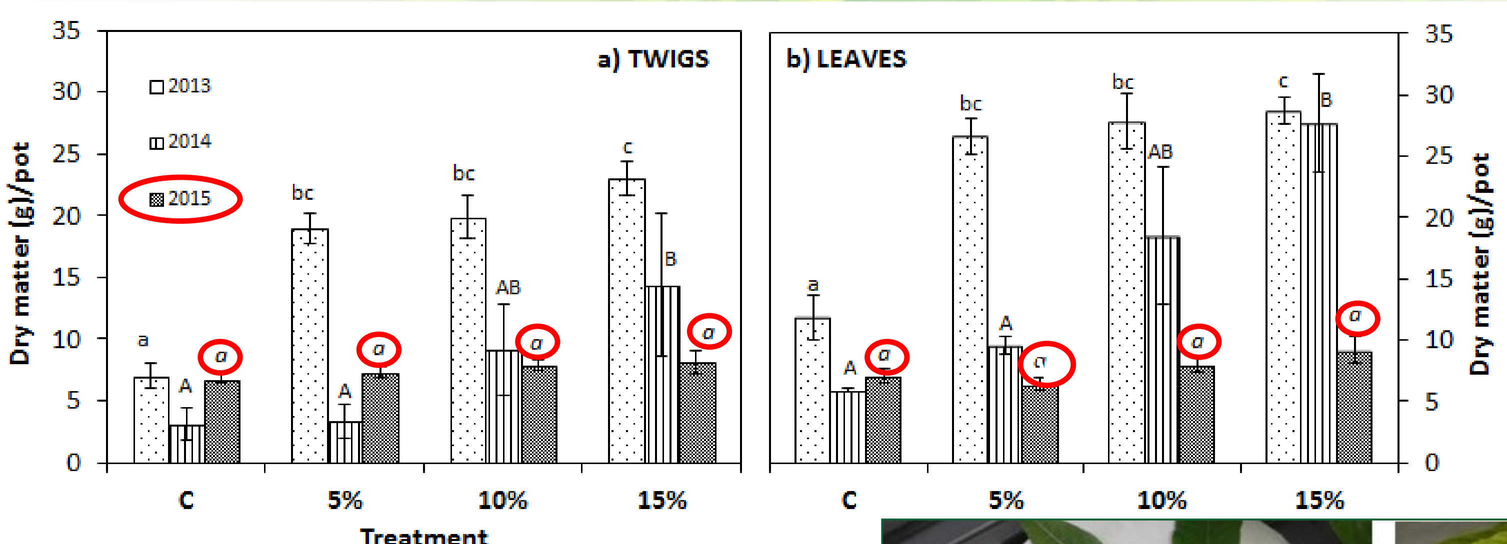


Phytostabilization - amendments

Extremely contaminated soil (Cd: 40 mg kg^{-1}; Zn: 5623 mg kg^{-1} ; Pb: 3706 mg kg^{-1})

- **3-years lyzimeter experiment: willows + biochar**
- 3 biochar rates applied (**5, 10, 15 %**)
- **Mineral fertilization (0.1 g N; 0.16 g P; 0.4 g K per 1 kg)**

Yield of willow biomass



Phytostabilization - amendments

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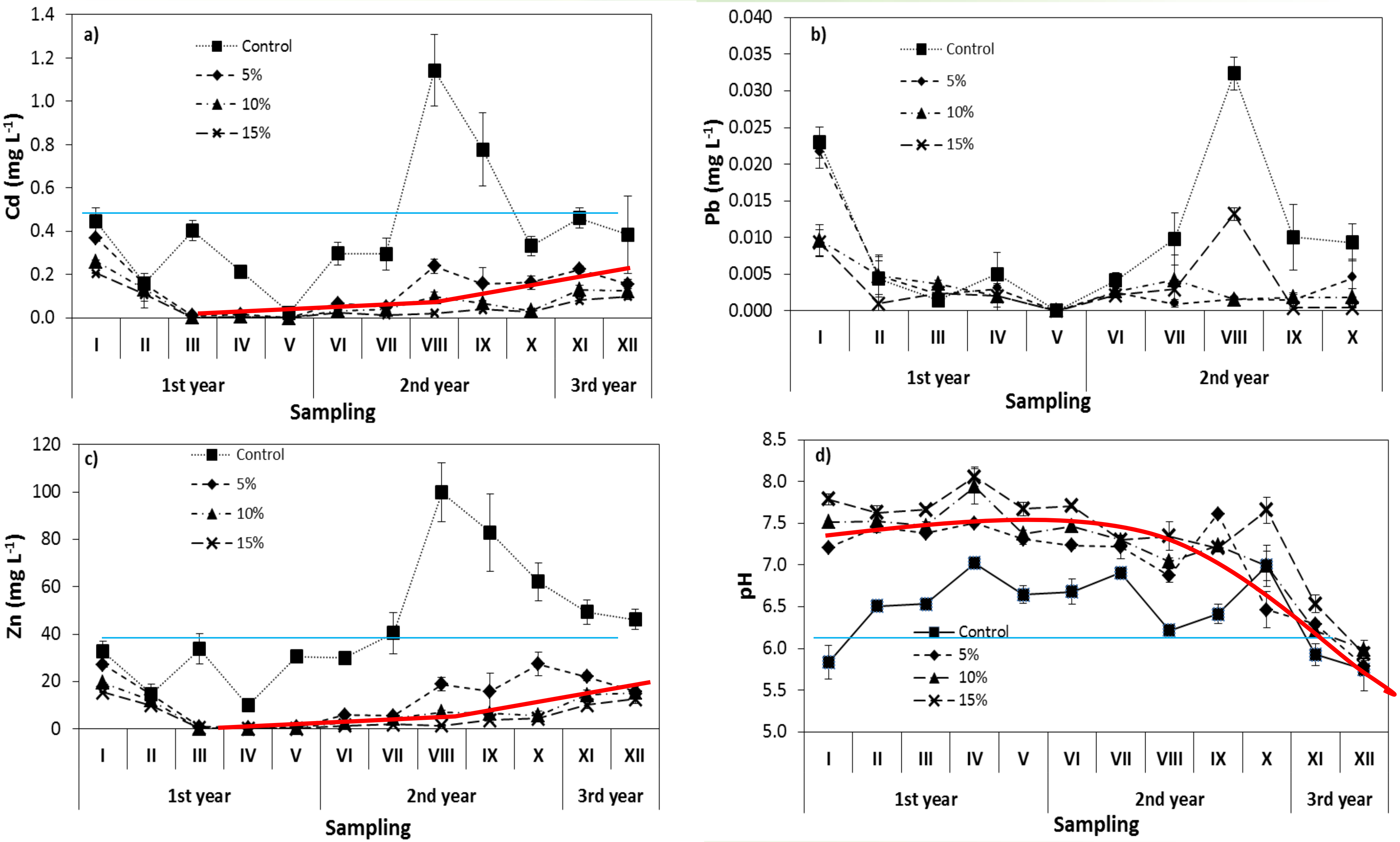
3-years lyzimeter experiment: willows + biochar

Leaching of metals and pH changes



Phytoextraction - Pot experiment Material and Methods

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Experimental design – pot experiment

- **7 years experiment**
- Fast-growing trees (*Salix smithiana, Populus nigra*).
- Three soils with different level of heavy metal contamination.

		Soil A	Soil B	Soil C
Total metal concentrations (mg kg^{-1})	Cd	**0.978 ± 0.109**	**2.23 ± 0.26**	**3.33 ± 0.18**
	Pb	56.5 ± 8.3	336 ± 15	454 ± 54
	Zn	**67.1 ± 7.9**	**152 ± 0**	**122 ± 4**
CEC ($mmol_+$ kg^{-1})		144	206	130
pH $_{(CaCl_2)}$		7.06	6.61	5.99
TOC (%)		2.0	2.3	1.8
Soil type		Modal Cambisol		

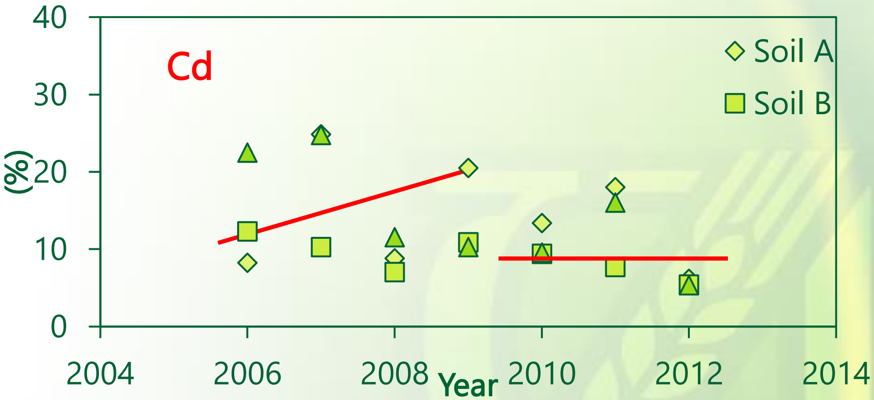
- Trees were planted in plastic pots (6 L, 20 cm diam.) contained 5 kg of soil (dry weight).
- Control treatments without plants were also set up.
- Precipitation-controlled outdoor vegetation hall (natural temperature and light conditions)
- Mineral fertilization (0.1 g N; 0.16 g P; 0.4 g K per 1 k

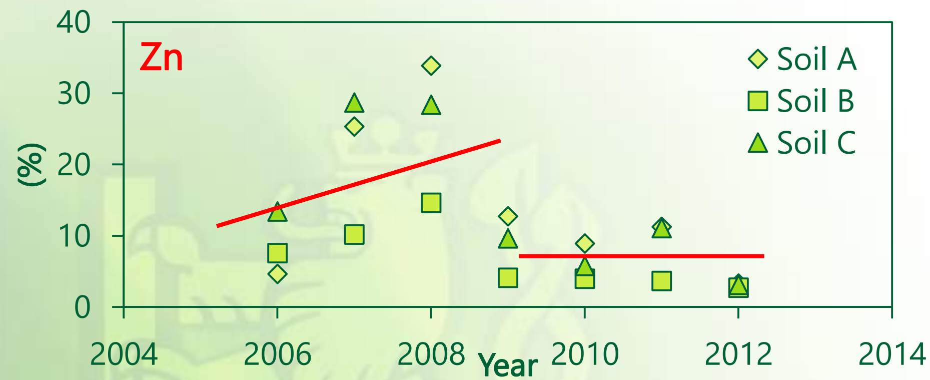
Phytoextraction - Pot experiment, Remediation factors

Remediation factors (RF) of harvestable plant parts during years (%)

cumulative remediation factor of 7 years

Salix smithiana





Cumulative remediation factors of biomass (%)				Annual (%)
Plant	Element	Harvestable	Non-harvestable	H + N
Salix s.	Cd	30.0	6.42	5,2
	Zn	15.4	1.76	2,5
Populus n.	Cd	26.7	0.95	4,0
	Zn	16.8	3.00	2,8

Phytoextraction - Pot experiment, Cadmium fractions distribution in soils after 7 vegetation periods

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		exchangeable		reducible		oxidizable		total
Plant	Soil	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}
Salix smithiana	A	0.16 ± 0.07	48	0.08 ± 0.07	24	0.08± 0.02	24	0.33± 0.04
	B	0.53 ± 0.04	34	0.87 ± 0.04	56	0.14± 0.00	9	1.55± 0.04
	C	1.01 ± 0.07	52	0.72 ± 0.13	37	0.14± 0.02	7	1.94± 0.18
Populus nigra	A	0.18 ± 0.01	45	0.14 ± 0.01	35	0.06± 0.01	16	0.40 ± 0.02
	B	0.65 ± 0.06	32	0.92 ± 0.09	45	0.16± 0.02	9	2.05± 0.05
	C	1.45 ± 0.05	51	1.22 ± 0.22	43	0.19 ± 0.02	7	2.86 ± 0.16
No plant	A	0.24 ± 0.03	39	0.32 ± 0.00	52	0.06 ± 0.00	10	0.62 ± 0.04
	B	1.09 ± 0.07	35	1.78 ± 0.05	58	0.22 ± 0.03	7	2.71±0.37
	C	1.10 ± 0.14	41	1.39 ± 0.45	45	0.22 ± 0.06	8	3.09± 0.28

The sequential extraction protocol was perfomed according to the original BCR procedure.

Phytoextraction - Pot experiment, Cadmium fractions distribution in soils after 7 vegetation periods

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Populus nigra	A	0.18 ± 0.01	45	0.14 ± 0.01	35	0.06± 0.01	16	0.40 ± 0.02
	B	0.65 ± 0.06	32	0.92 ± 0.09	45	0.16± 0.02	9	2.05± 0.05
	C	1.45 ± 0.05	51	1.22 ± 0.22	43	0.19 ± 0.02	7	2.86 ± 0.16
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	C	1.10 ± 0.14	41	1.39 ± 0.45	45	0.22 ± 0.06	8	3.09± 0.28

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Phytoextraction - Field experiment - Material and methods

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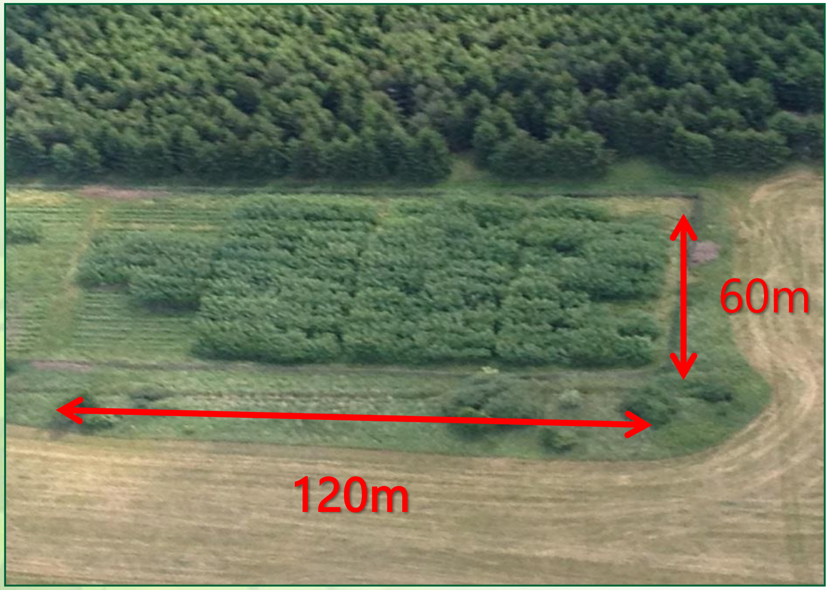
60 km S of Prague, the Czech Republic: Příbram – Podlesí

Level of contamination: medium; antrophogenic

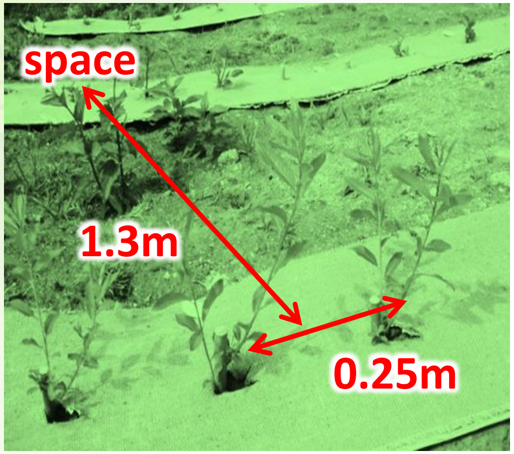
Soil parameters		
Total metal concentrations (mg kg^{-1})	Cd	8.3±1.2
	Pb	1214±99
	Zn	218±26
CEC ($mmol_+ kg^{-1}$)		166
pH (KCl)		5.27
TOC (%)		4.1
Soil type		Modal Cambisol



Fast-growing trees - planted in April 2008:

willows (S1: Tordis; S2: S. smithiana S-218),

poplars (P1: (*P. nigra × P. maximowiczii*) J 105; P2: Wolterson)

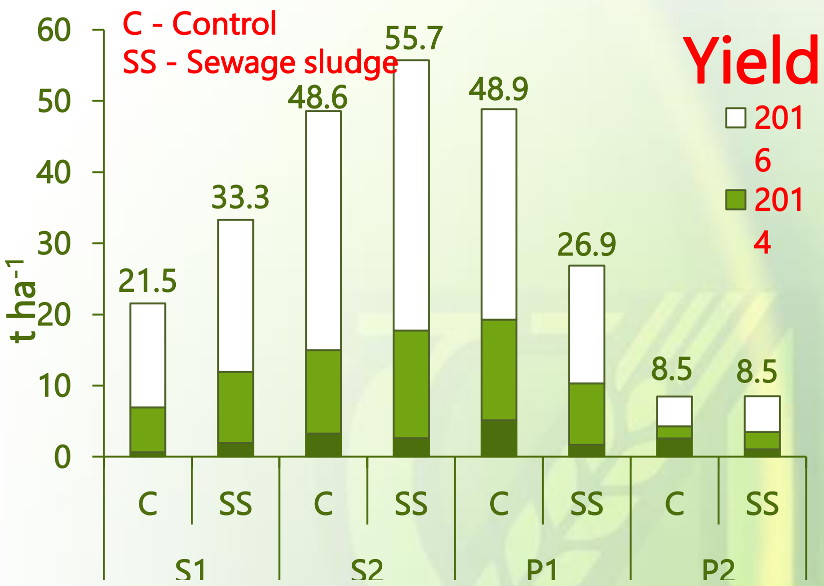


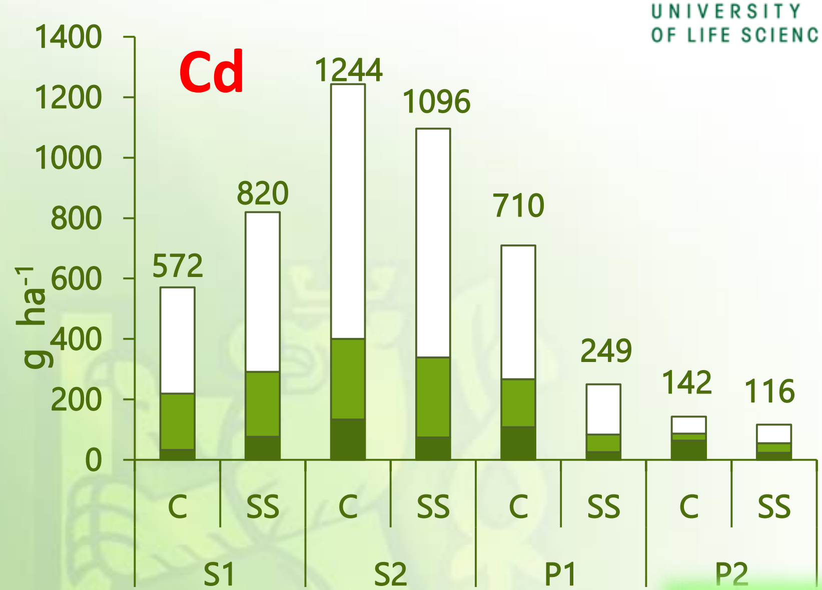
Harvests: 1st (4 years) 2012 | 2nd (next 2 years) 2014 | 3rd: (next 2 years) 2016

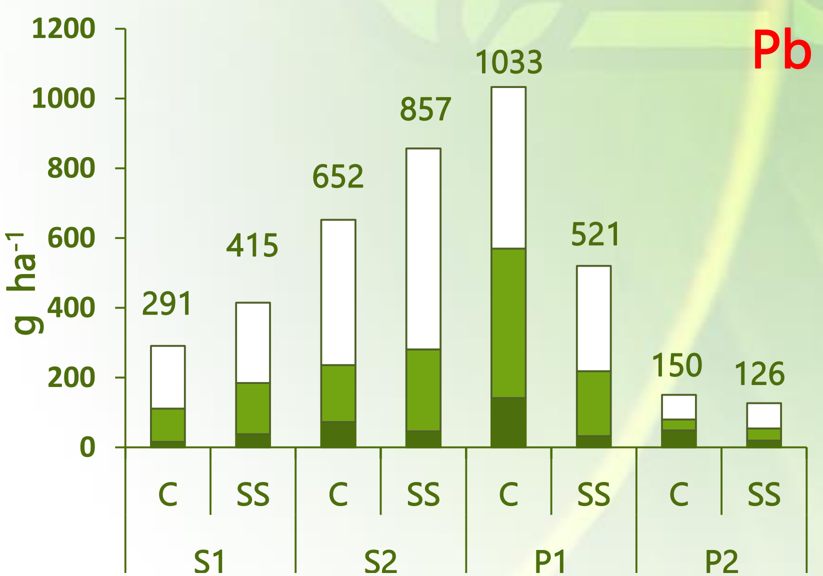
Sewage sludge application

Year	Rate	N	P	Cd	Pb	Zn
2008	kg ha^{-1}	367	71	0.03	0.92	8
2012		323	66	0.01	0.29	4

Phytoextraction - Field experiment - Biomass yield (t ha^{-1}) and element removal (g ha^{-1}) - three harvests







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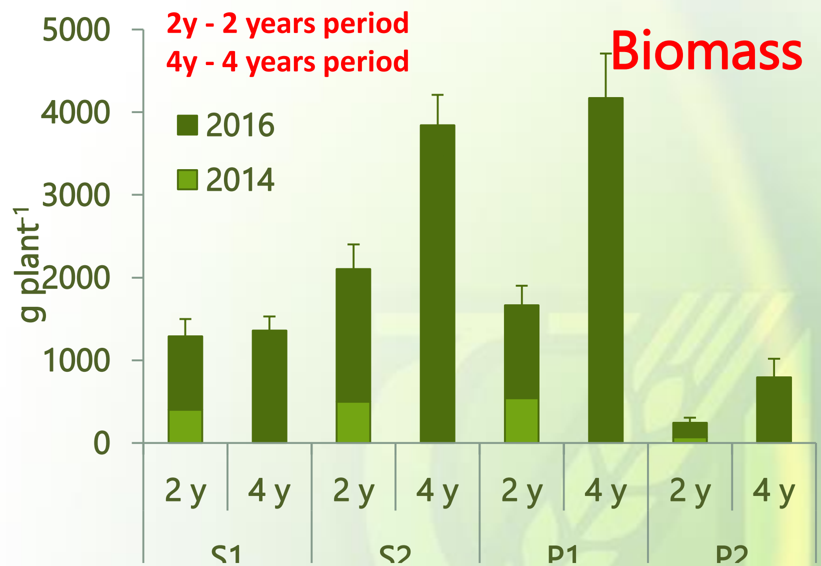
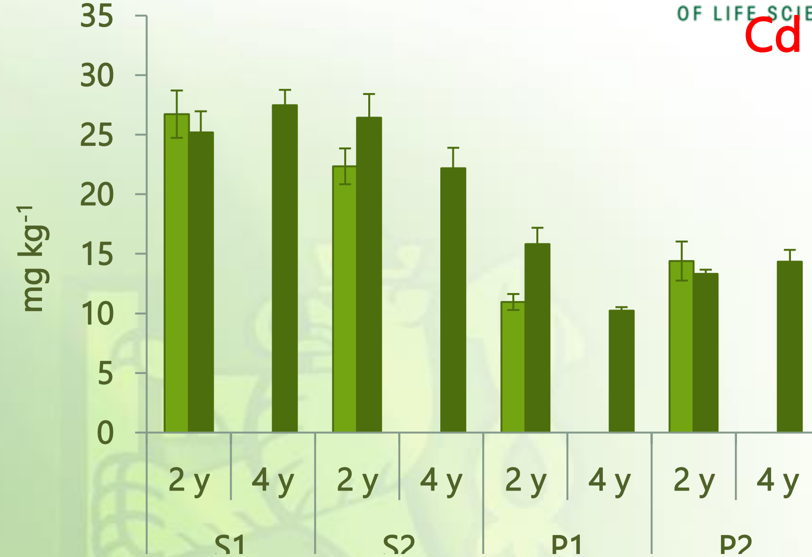
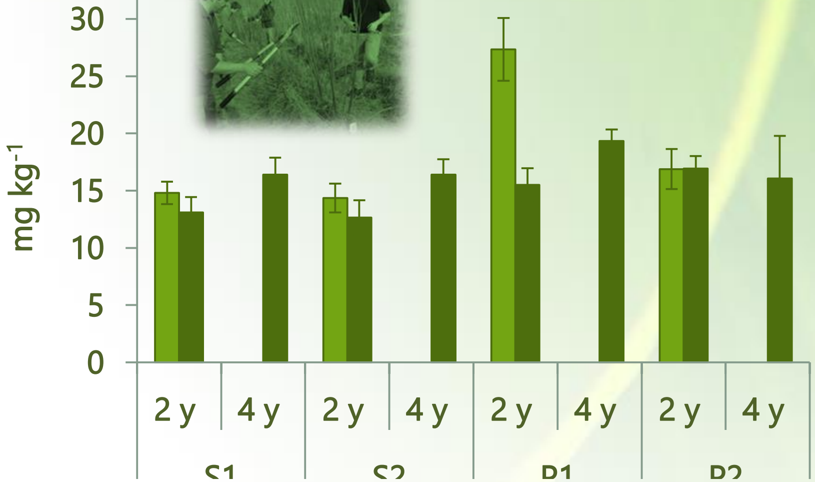
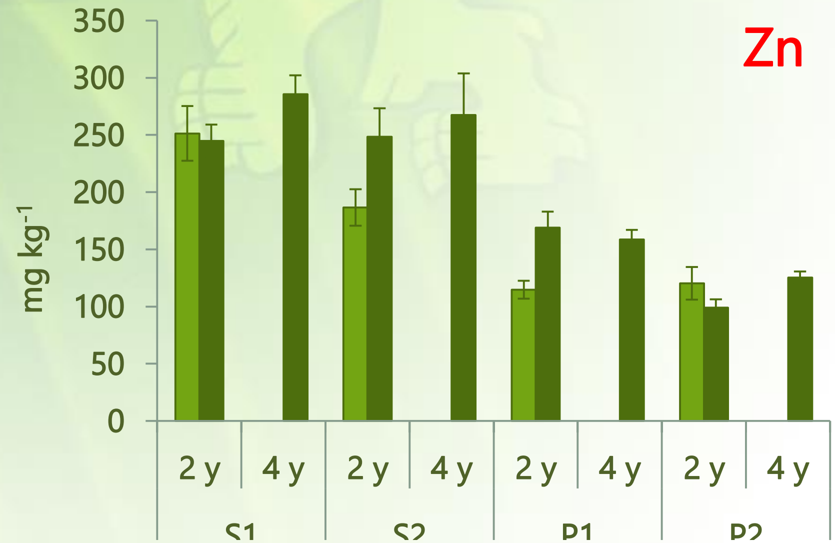
Phytoextraction - Field experiment - Biomass production (g plant^{-1}) and element content (mg kg^{-1}) - two and four years harvest periods









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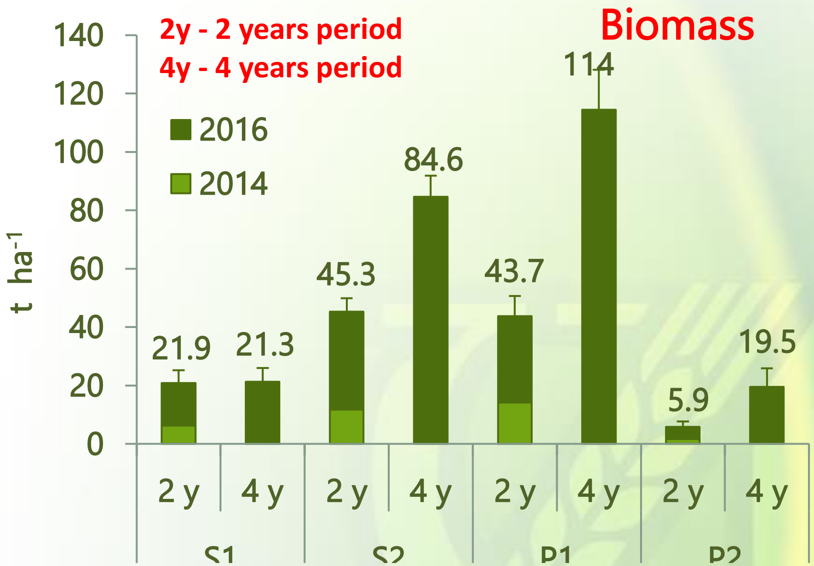
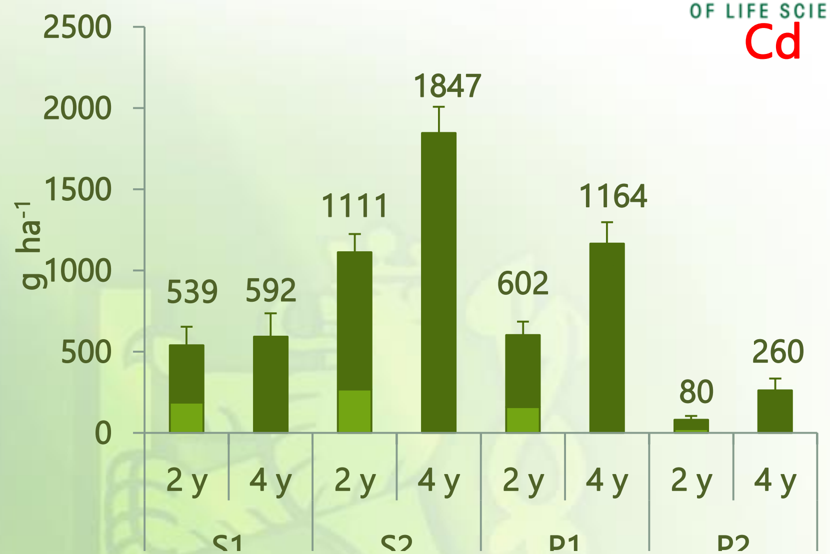
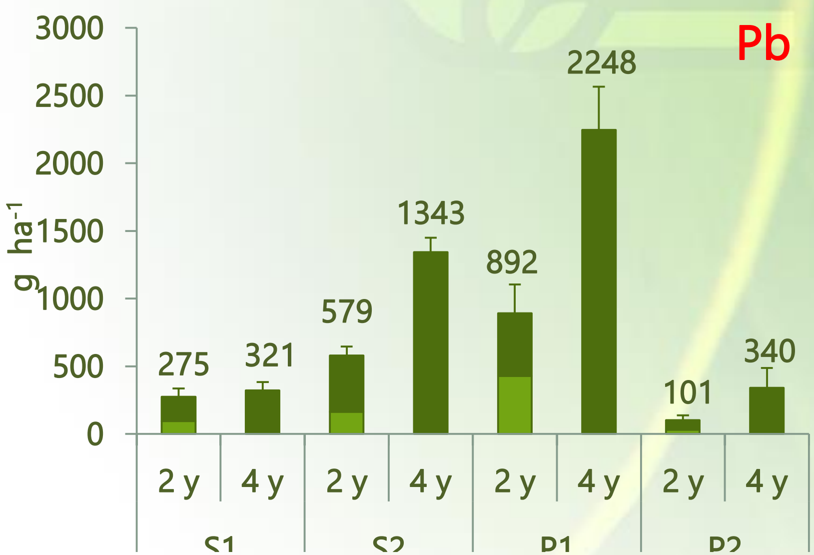
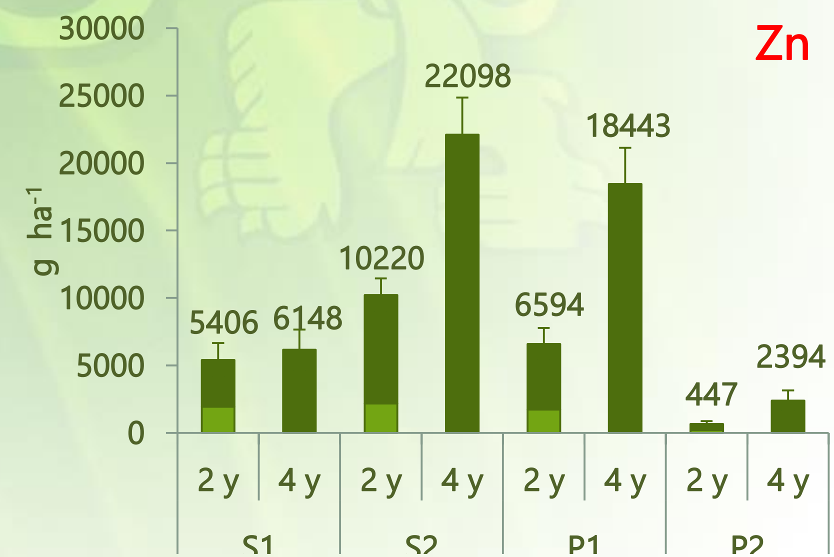
Phytoextraction - Field experiment - Biomass yield (t ha^{-1}) and element removal (g ha^{-1}) - two and four years harvest periods









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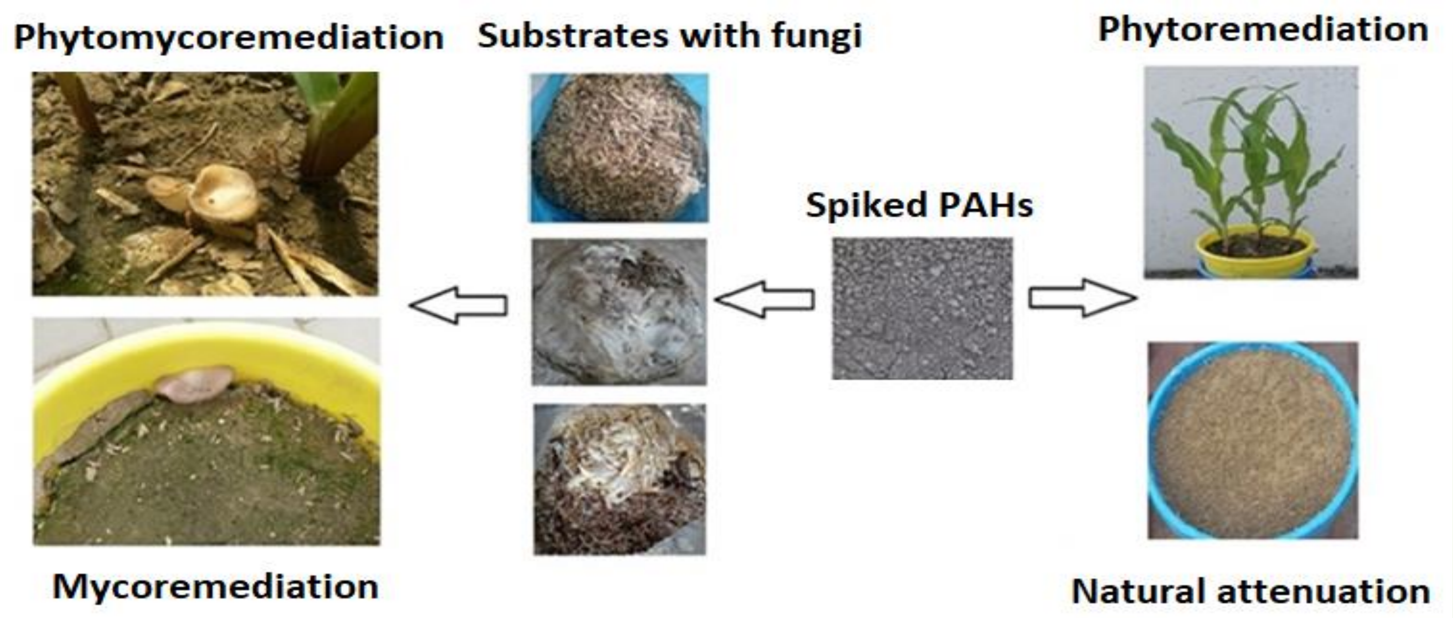
Phyto/mycoremediation of soil contaminated by spiked PAHs



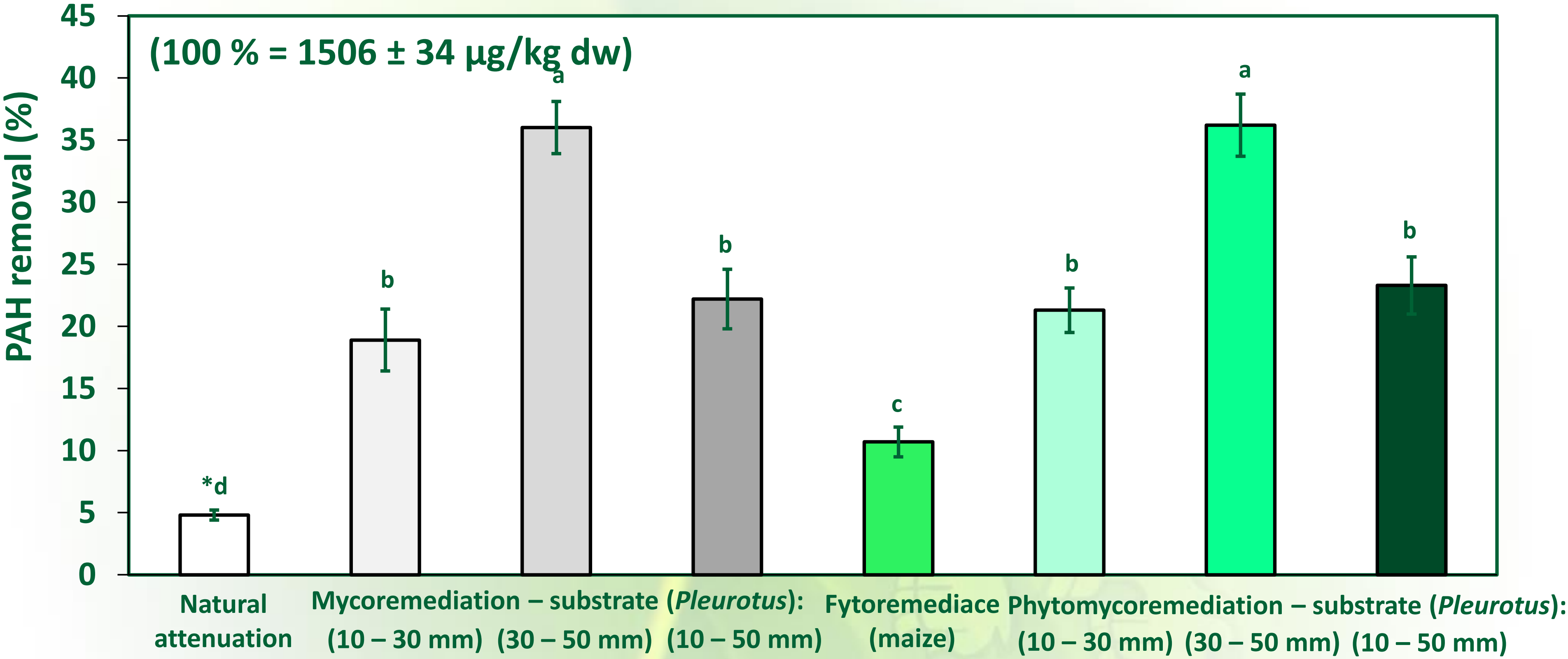
- 5 kg soil + NPK/pot; plants: maize (*Zea mays* L.)
- amendment/pot: 5 % (*w*/*w*) **oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm., HK35) grown on wooden chips**

Phyto/mycoremediation of aged PAHs in soil

- 5 kg soil + NPK/pot (loam sandy; gleyic Fluvisol; pH($CaCl_2$) = 7.5; CEC = 76 $mmol_+$/kg); plants: maize (*Zea mays* L.)
- amendment/pot: 6 % (*w*/*w*) of **ligninolytic fungi (*Crucibulum laeve* Huds.*)* grown on barley seeds**

Phytodegradation and Bioremediation

- Phyto/mycoremediation of soil contaminated by spiked PAHs

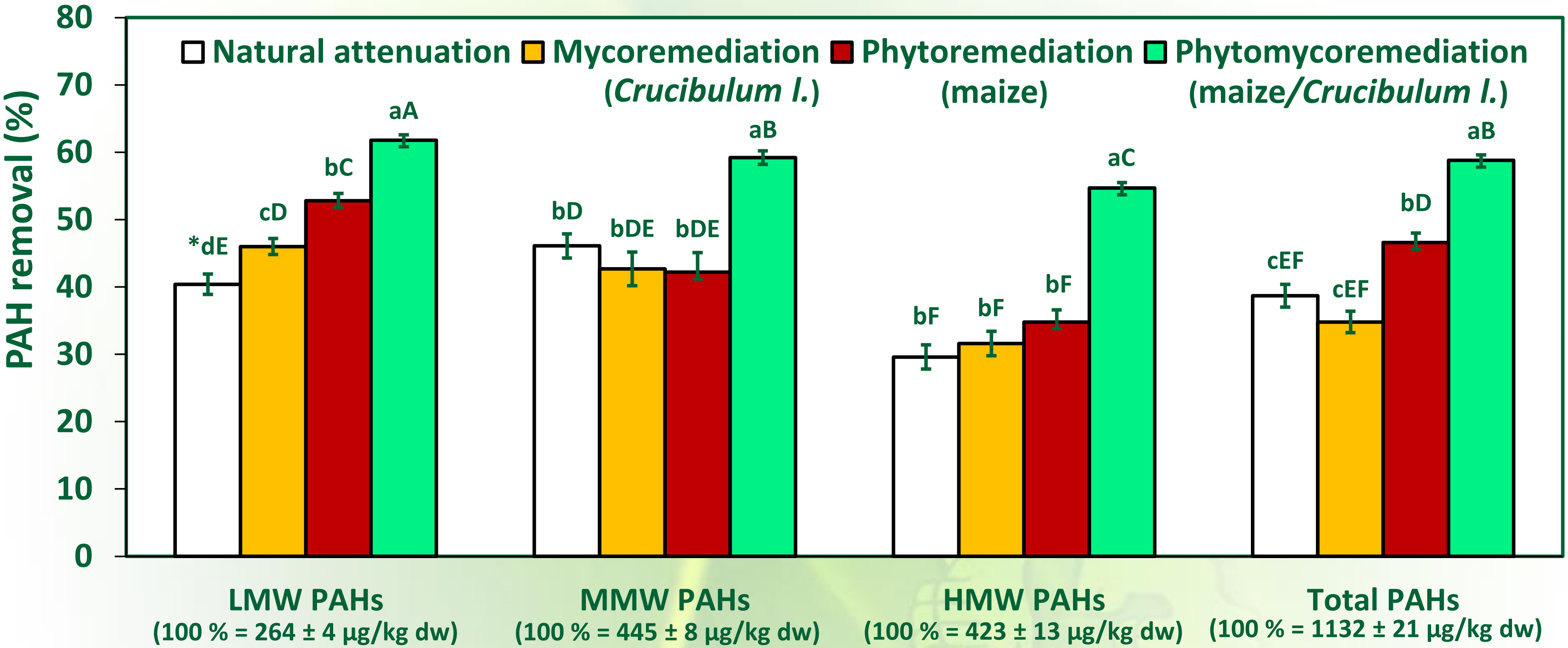


	Natural attenuation	Mycoremediation - Substrate with fungi (*Pleurotus o.*):			Phytoremediation	Phytomycoremediation - Substrate with fungi (*Pleurotus o.*):		
		10-30 mm	30-50 mm	10-50 mm		10-30 mm	30-50 mm	10-50 mm
Fungi biomass (µg ergosterol/g soil)	7,8 ± 3,3[c]	48,0 ± 3,2[ab]	45,1 ± 3,2[b]	41,9 ± 3,9[b]	37,3 ± 5,1[b]	66,5 ± 5,1[a]	65,8 ± 15[a]	54,4 ± 12[ab]
Manganase peroxidase (U/g soil dw)	0,4 ± 0,1[c]	0,5 ± 0,1[bc]	0,9 ± 0,3[b]	0,5 ± 0,0[bc]	0,6 ± 0,2[bc]	0,5 ± 0,0[bc]	1,5 ± 0,3[a]	0,5 ± 0,0[bc]

*Small letters indicate statistical differences (P < 0,05) between enzymeactivities in various treatments.

Phytodegradation and Bioremediation

- **Phyto/mycoremediation of aged PAHs in soil**



	Natural attenuation	Mycoremediation	Phytoremediation	Phytomycoremediation
Microbial activity (µg TPF/g soil dw / 24 h)	197 ± 10^{C}	$387 \pm 6{,}2^{B}$	$392 \pm 5{,}3^{B}$	$437 \pm 4{,}8^{A}$
Fungi biomass (µg ergosterol/g soil dw)	$86 \pm 3{,}4^{D}$	$141 \pm 6{,}3^{B}$	$122 \pm 4{,}3^{C}$	$162 \pm 8{,}6^{A}$

*Capital letters indicate statistical differences (P < 0,05) between enzyme activities in various treatments.

Phytoremediation of soil PAHs using organic amendments

- organic amendments from organic waste mixture (pH(H_2O) = 8.4; C = 38.7 %; N = 2.1 %)
- amendment/pot: 10 % (w/w) compost or vermicompost; 1 % (w/w) fly ash
- 6 bare and 6 planted treatments (n = 4):
- 1. control, 2. compost, 3. vermicompost, 4. ash, 5. compost + ash, 6. vermicompost + ash

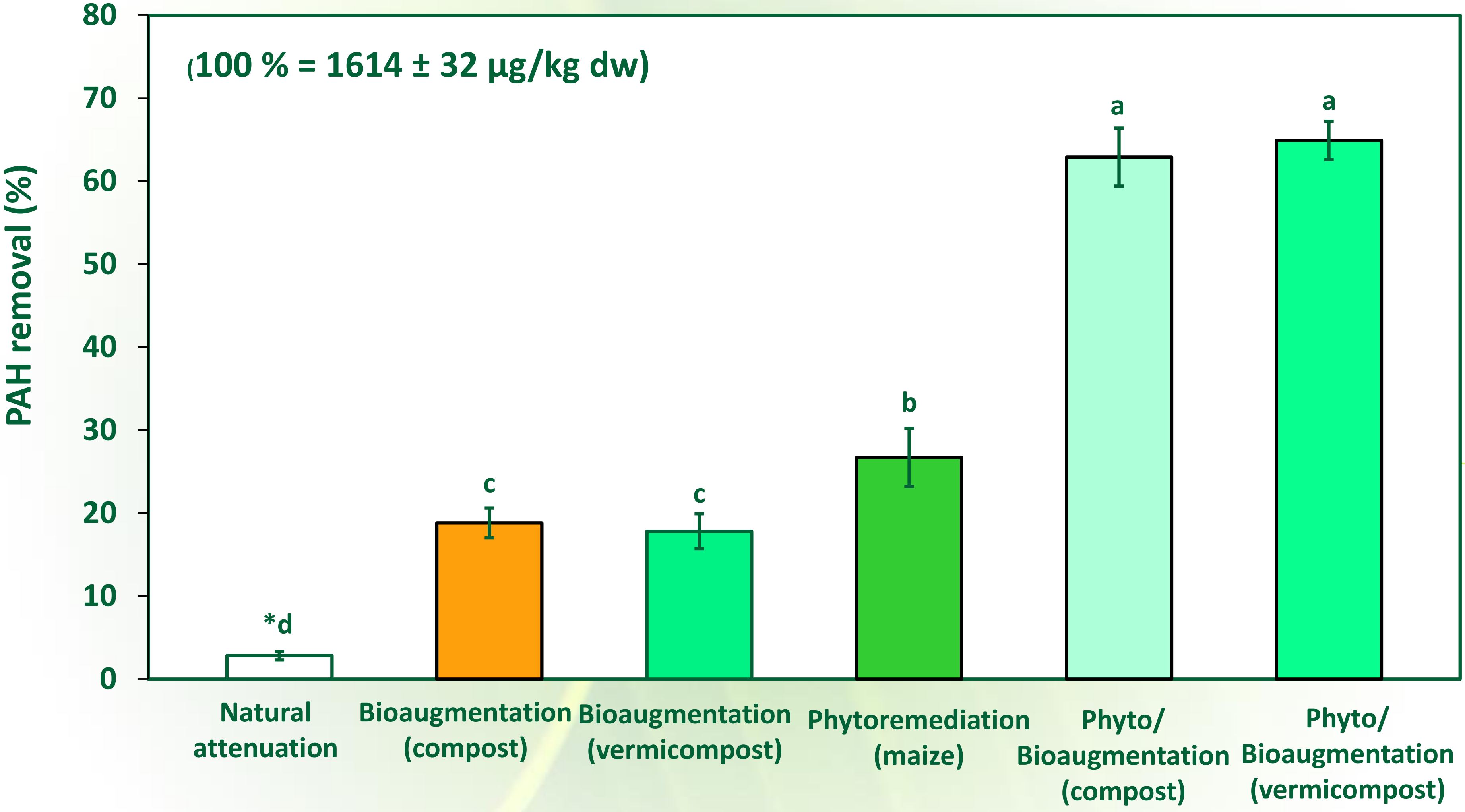
Bioremediation of PAHs through the composting and vermicomposting

- 4 treatments
- amendment/organic mixture: a) 5 % (*w*/*w*) fly ash
  b) spiked PAHs (~500 µg/kg dw)

- <u>composting:</u> fermentors – forced aeration
- <u>vermicomposting:</u> vermireactors
  - earthworms of genus *Eisenia andrei* (Bouché)

Phytodegradation and Bioremediation

- **Phytoremediation of soil PAHs using compost and vermicompost**

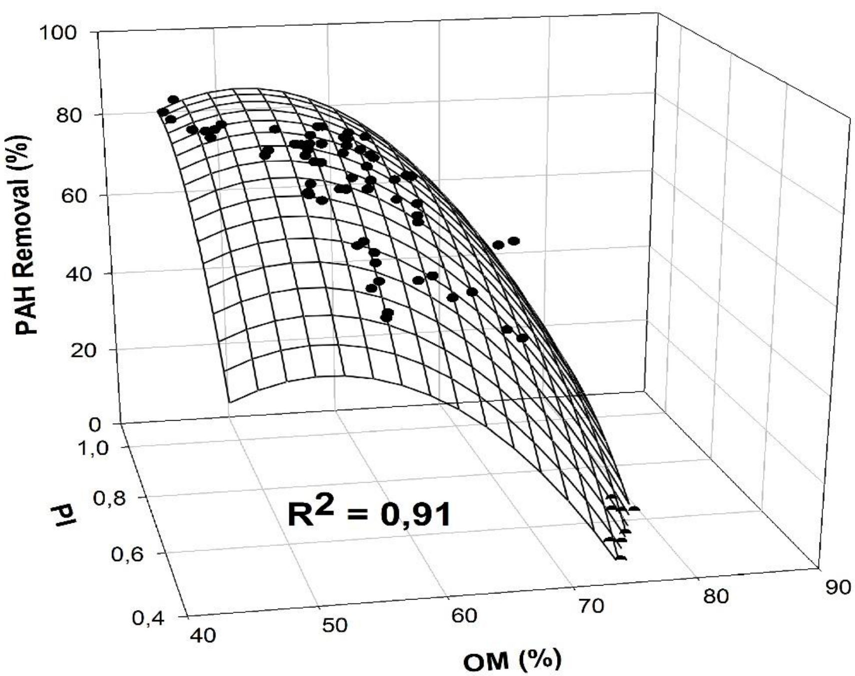


*Small letters indicate statistical differences (P < 0,05) between PAHs in various treatments.

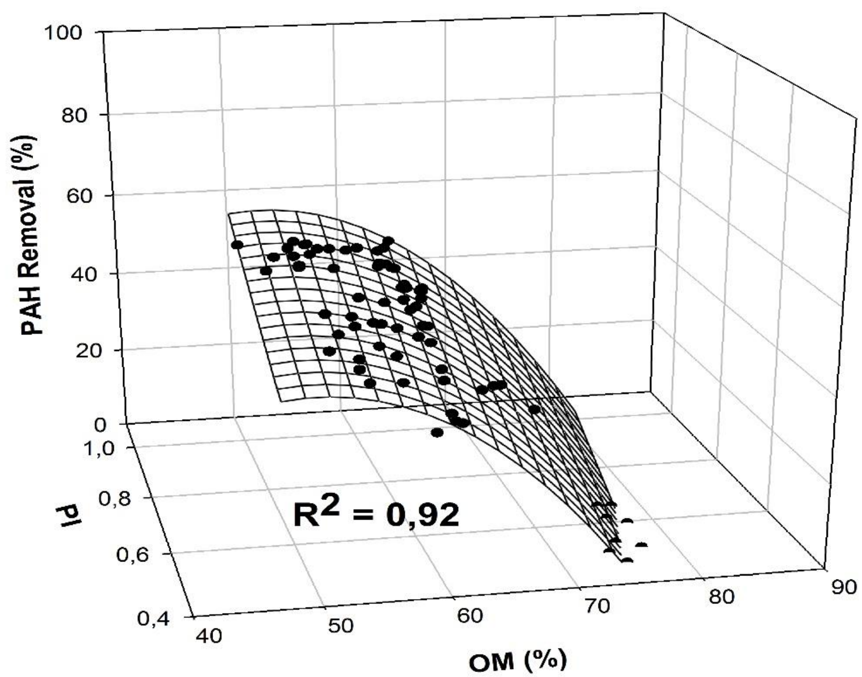
Bioremediation – composting and vermicomposting

- **Biodegradation of PAHs through the composting and vermicomposting**

a) Composting



b) Vermicomposting



(PI = Polarity Index)

(100 % PAH content = 6849 ± 97 μg/kg dw)

Enzyme activities	Composting	Vermicomposting
Lipase (U/g soil dw)	7732 ± 1732[aA]	1 998 ± 397[bA]
Manganase peroxidase (mU/g soil dw)	1,4 ± 0,1[aB]	0,5 ± 0,1[bB]

*Small letters indicate statistical differences ($P < 0,05$) between enzyme activities in respected treatment.
Capital letters indicate statistical differences ($P < 0,05$) between enzyme activities in various treatments.

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Conclusion Phytostabilization and Phytoextraction

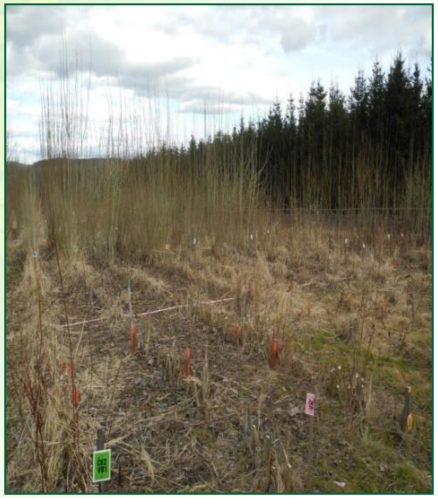
Extremely contaminated soil

Soil amendments are necessary to immobilize toxic metals **(Zn)** to allow plants to grow sufficiently.

Low –medium contaminated soil

Only **"mobile"** elements (**Cd, Zn**)can be removed by plants

- Choice of proper clone is crucial to get success
- Biomass yield is a driving force for successful phytoextraction
- Mean annual removal was higher by willow than poplar



Element	Pot (%) /7y/	Field (%) /8y/	Field (%) /4y/
Cd	5.2	1.6	3.1
Zn	2.5	0.7	1.3

- The length of experiment supports element extraction
- Application of organic fertilizers did not increase remediation efficiency
- Longer interval between harvests increased element removal

Conclusion - Phytodegradation and Bioremediation

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1) Bioaugmentation

– **average removal of total PAHs was 15 %**

2) Bioaugmentation

– compost, vermicompost : **average PAHs removal was 24 %**

3) Phytoremediation

– maize: **average removal of total PAHs was 27 %**

4) Phytoremediation assisted by mushroom substrates

– maize + *Pleurotus o.:* **average removal of total PAHs was 36 %**

– maize + *Crucibulum l.*: **average removal of total PAHs was 58 %**

5) Phytoremediation assisted by organic materials

– maize + compost nebo vermicompost: **average removal of total PAHs was 64 %**

6) Vermicomposting

– **average removal of total PAHs was 60 %**

7) Composting

– **average removal of total PAHs was 82 %**

Using maize plant in bioremediation:

maize could be used in PAH bioremediation especially in combination with organic amendments

harvested above ground biomass has not to be landfilled

Using composting and vermicomposting in PAH bioremediation

resulted composts or vermicomposts could be used eg. as soil organic amendments

Thank you for your attention!

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