



Risultati degli esperimenti in serra: PCB, Diossine/Furani, Arsenico e Mercurio



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Contesto e obiettivi

- ❖ **Accordo di programma** tra MATTM ed enti territoriali coinvolti per messa in sicurezza e bonifica del SIN Brescia-Caffaro (settembre 2009).
- ❖ Progettazione della **bonifica dei terreni** delle aree agricole del **SIN Brescia-Caffaro** affidata a Regione Lombardia (e a sua volta ad ERSAF) (marzo 2013).
- ❖ Sperimentazione di tecnologie di **bioremediation** su aree agricole.



3 aree di circa 5 ettari (**A**, **R**, **T**),
per una superficie complessiva
di circa 12 ettari

 Confine aree agricole SIN

Perchè in serra e non in campo?



- In letteratura molti lavori con diverse specie di piante e condizioni.
- Necessità di selezionare le specie/condizioni migliori per il suolo del SIN-Brescia Caffaro.
- Alta variabilità spaziale delle concentrazioni.
- Necessità di selezionare una concentrazione costante ed omogenea.
- Tuttavia adottare un disegno sperimentale che garantisse delle condizioni di “semi-campo”.
- Budget e tempo limitato per il progetto.

Selezione specie e condizioni

Consultazione di più di **100 lavori scientifici**



Compilazione di una **tabella riassuntiva**



Stesura di un ***background document***



Definizione di **un protocollo**





Review

Phyto-rhizoremediation of polychlorinated biphenyl contaminated soils: An outlook on plant-microbe beneficial interactions



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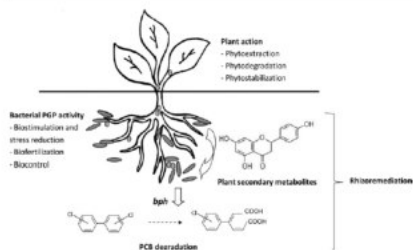
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HIGHLIGHTS

- Rhizoremediation is the most promising biotechnology for PCB-polluted soils.
- Root exudates can induce the biphenyl catabolic pathway enhancing degradation ability.
- Better knowledge on *in situ* phyto-rhizoremediation of PCB-polluted soils is needed.
- "Omics" can unravel key players and metabolisms for remediation of PCB-polluted sites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form 28 September 2016
Accepted 28 September 2016
Available online 5 October 2016

Editor: D. Barcelo

Keywords:
Phytoremediation
Rhizoremediation
Polychlorinated biphenyls (PCBs)
Plant growth promoting bacteria
Endophytes
Root exudates

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<http://dx.doi.org/10.1016/j.scitotenv.2016.09.218>
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ABSTRACT

Polychlorinated biphenyls (PCBs) are toxic chemicals, recalcitrant to degradation, bioaccumulative and persistent in the environment, causing adverse effects on ecosystems and human health. For this reason, the remediation of PCB-contaminated soils is a primary issue to be addressed. Phytoremediation represents a promising tool for *in situ* soil remediation, since the available physico-chemical technologies have strong environmental and economic impacts. Plants can extract and metabolize several xenobiotics present in the soil, but their ability to uptake and mineralize PCBs is limited due to the recalcitrance and low bioavailability of these molecules that in turn impedes an efficient remediation of PCB-contaminated soils. Besides plant degradation ability, rhizoremediation takes into account the capability of soil microbes to uptake, attack and degrade pollutants, so it can be seen as the most suitable strategy to clean-up PCB-contaminated soils. Microbes are in fact the key players of PCB degradation, performed under both aerobic and anaerobic conditions. In the rhizosphere, microbes and plants positively interact. Microorganisms can promote plant growth under stressed conditions typical of polluted soils. Moreover, in this specific niche, root exudates play a pivotal role by promoting the biphenyl catabolic pathway, responsible for microbial oxidative PCB metabolism, and by improving the overall PCB degradation performance. Besides rhizospheric microbial community, also the endophytic bacteria are involved in pollutant degradation and



Review

Rhizoremediation half-lives of PCBs: Role of congener composition, organic carbon forms, bioavailability, microbial activity, plant species and soil conditions, on the prediction of fate and persistence in soil



Elisa Terzaghi ^a, Elisabetta Zanardini ^a, Cristiana Morosini ^a, Giuseppe Raspa ^b, Sara Borin ^c, Francesca Mapelli ^c, Lorenzo Vergani ^c, Antonio Di Guardo ^{a,*}

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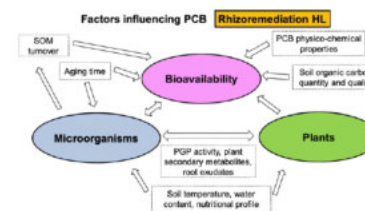
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HIGHLIGHTS

- PCB bioavailability influences their persistence in soil.
- Plant-microbe beneficial interactions could decrease PCB persistence in soil.
- PCB half-lives in soil reported in the literature range from ~1 to >20 years.
- PCB rhizoremediation half-lives in soil range from ~50 days to ~11 years.
- Comparable rhizoremediation experiments are needed to obtain robust data.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 July 2017
Received in revised form 17 August 2017
Accepted 17 August 2017
Available online xxxxx

Editor: D. Barcelo

Keywords:
Polychlorinated biphenyls (PCBs)
Half-life
Rhizoremediation
Bioavailability
Modelling
Plant

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<http://dx.doi.org/10.1016/j.scitotenv.2017.08.189>
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Criteri per scelta specie

- Effetto sulla degradazione di PCB (PCDD/F)
- Capacità fitoestrattive per metalli
- Caratteristiche botaniche
- Esigenze ambientali
- Numerosità studi per specie
- Rilevanza (IF) della rivista scientifica



Definizione protocollo

- Specie vegetali
- Trattamenti
- Controlli
- Repliche
- Tempi
- Condizioni sperimentali
- Tipo e numerosità matrici
- Tipo di analisi



Disegno sperimentale

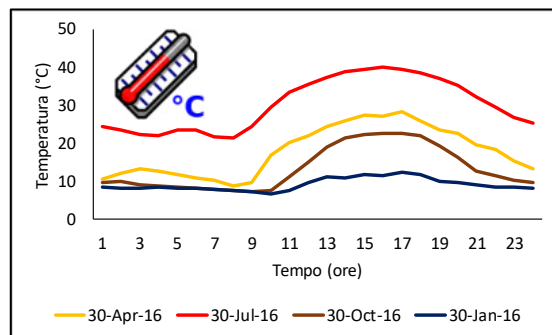
- ❖ **TRATTAMENTI – CONTROLLI – REPLICHE:** 10 trattamenti + 7 controlli, tutti in triplicato
- ❖ **DURATA ESPERIMENTO:** Maggio 2015 – Novembre 2016 (circa 18 mesi)
- ❖ **TEMPI DI CAMPIONAMENTO:** T0, T1 (3 mesi), T2 (6 mesi), T3 (12 mesi), T4 (18 mesi)
- ❖ **MATRICI CAMPIONATE:** suolo e biomassa vegetale (radici e foglie)
- ❖ **CONTAMINANTI RICERCATI:** PCB, PCDD/F, DDX, As e Hg
- ❖ **ANALISI MICROBIOLOGICHE:** profilazione batteri e funghi e test attività microbica

Attivazione dei microrganismi del suolo durante la sperimentazione – **Sara Borin** (Università degli Studi di Milano).

CONDIZIONI DI “SEMI-CAMPO”



25 x 20 cm (6 kg)



Piante e condizioni suolo: trattamenti

PIANTE

1. *Phalaris arundinacea* (scagliola palustre)
2. *Festuca arundinacea* (festuca falascona)
3. *Cucurbita pepo ssp pepo* (zucca)
4. *Medicago sativa* (erba medica)
5. *Brassica juncea* (senape indiana)
6. *Salix caprea* (salicone)
7. *Athyrium filix-foemina* (felce)

CONDIZIONI SUOLO

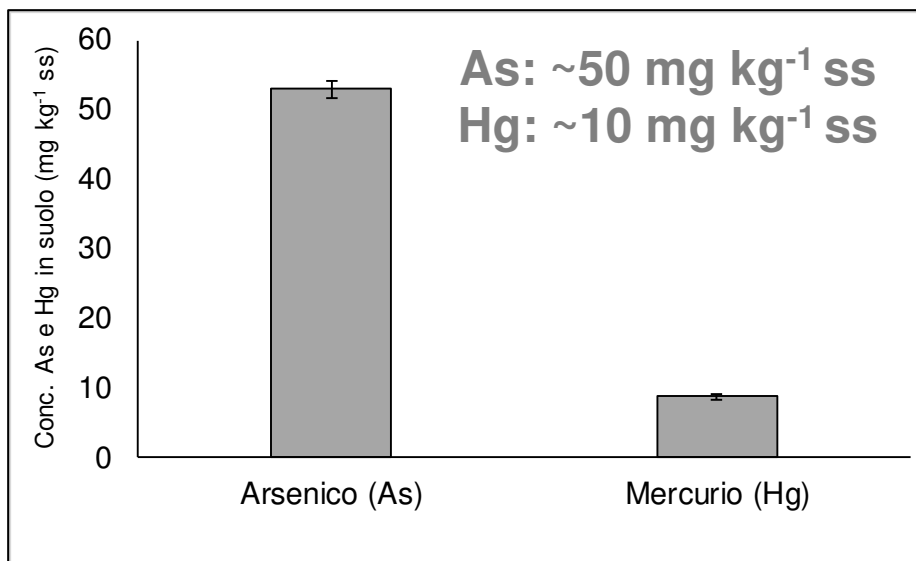
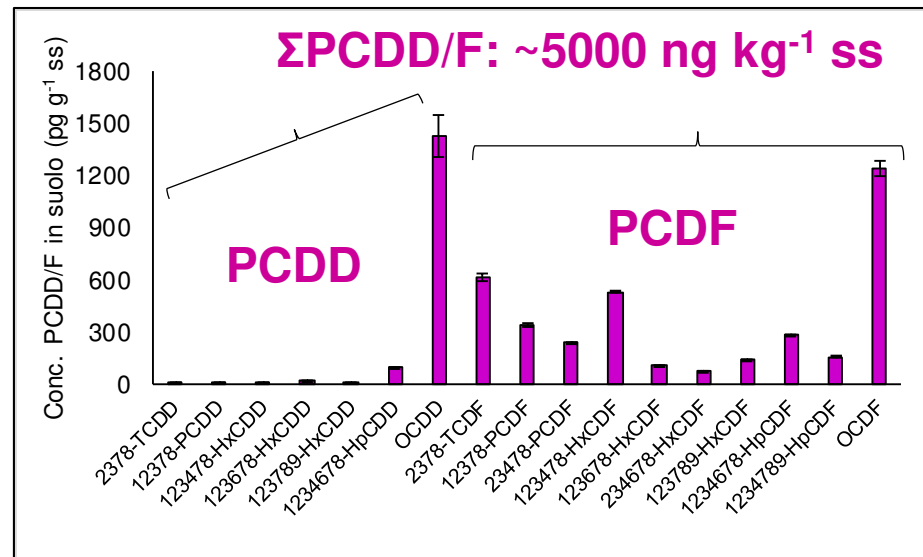
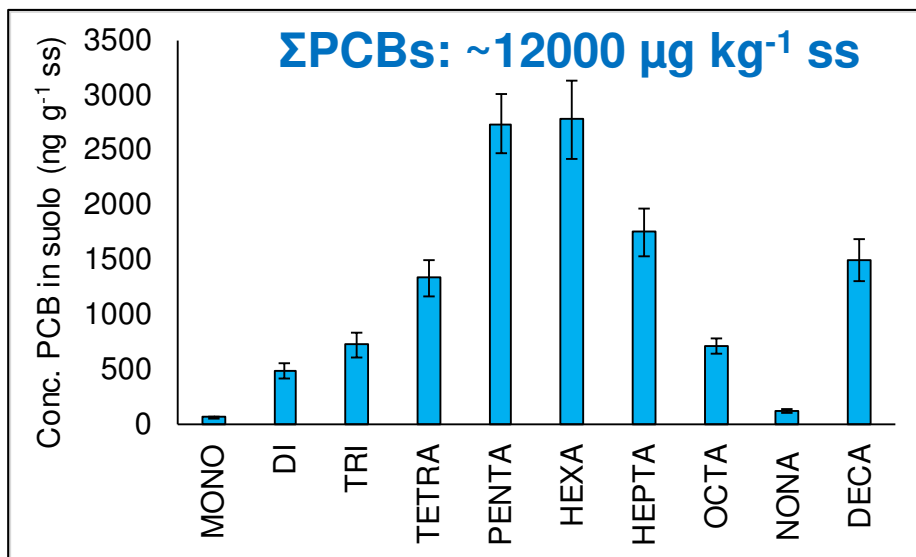
1. solo pianta
2. ciclo redox
3. consociazione
4. aggiunta compost
5. aggiunta tiosolfato

Piante e condizioni suolo: trattamenti

Specie	Trattamento(P)
<i>Phalaris arundinacea</i>	P1: solo pianta
	P2: cicli di irrigazione alternati
<i>Festuca arundinacea</i>	P3: solo pianta
	P4: consociazione con <i>Cucurbita pepo</i> ssp <i>pepo</i>
	P5: cicli di irrigazione alternati
	P6: aggiunta di compost
<i>Medicago sativa</i>	P7: aggiunta di rizobio e funghi micorrizici
<i>Salix caprea</i>	P8: consociazione con <i>Athyrium filix foemina</i>
<i>Brassica juncea</i>	P9: solo pianta
	P10: aggiunta tiosolfato d'ammonio

10 TRATTAMENTI (P) + 7 CONTROLLI (C)

Concentrazioni iniziali: T0 (maggio 2015)



**Concentrazioni finali:
T4 (novembre 2016)?**



Rhizoremediation of weathered PCBs in a heavily contaminated agricultural soil: Results of a biostimulation trial in semi field conditions

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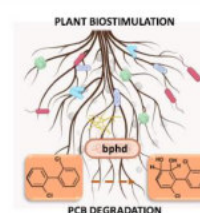
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HIGHLIGHTS

- Plants stimulated microbial diversity abundance and activity in root surrounding soil.
- Best degrader treatment was tall fescue with compost also increasing bacterial abundance.
- Depletion of both low and high chlorinated PCB was observed.
- Tall fescue and pumpkin treatments reduced about 40 PCB congeners.
- Overall total PCB reduction was about 20% in realistic semi-field conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 18 April 2019

Received in revised form 28 May 2019

Accepted 30 May 2019

Available online 05 June 2019

Editor: Damia Barcelo

Keywords:

Plants
Natural attenuation
Biodegradation
Greenhouse
Bioavailability
Contaminated site

ABSTRACT

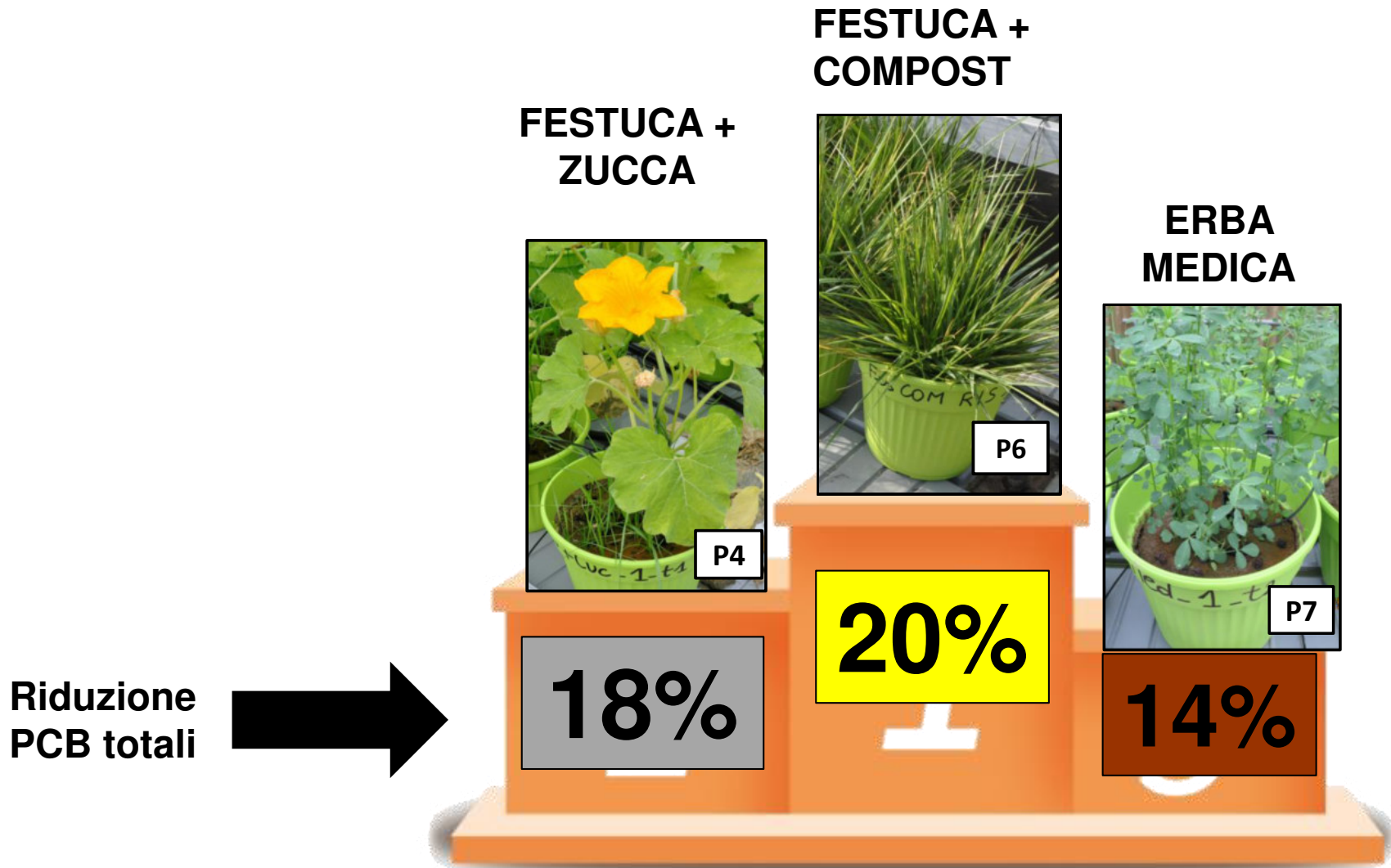
This paper describes the results of a rhizoremediation greenhouse experiment planned to select the best plant species and soil management for the bioremediation of weathered polychlorinated biphenyls (PCBs). We evaluated the ability of different plant species to stimulate activity and diversity of the soil microbial community leading to the reduction of PCB concentrations in a heavily contaminated soil (at mg kg⁻¹ dw level), of the national priority site for remediation (SIN) "Brescia-Caffaro" in Italy. Biostimulation was determined in large size (6 kg) pots, to reflect semi-field conditions with a soil/root volume ratio larger than in most rhizoremediation experiments present in the literature. In total, 10 treatments were tested in triplicates comparing 7 plant species (grass and trees) and 5 soil/cultivation conditions (i.e., only one plant species, plant consociation, redox cycle, compost or ammonium thiosulfate addition) with the appropriate unplanted controls. After 18 months of biostimulation the overall reduction of total PCBs varied between 14 and 20%. Microbial analysis revealed a shift in the microbial community structure over time and showed that all the planted treatments significantly enhanced microbial hydrolytic activity and the abundance of bacterial populations, including potential PCB degraders, in the soil surrounding plant roots. The plant species most effective in reducing the contaminant concentrations were *Festuca arundinacea* cultivated adding compost or in consociation with *Cucurbita pepo* ssp.

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¹ Shared first authors.

Risultati PCBs: trattamenti migliori



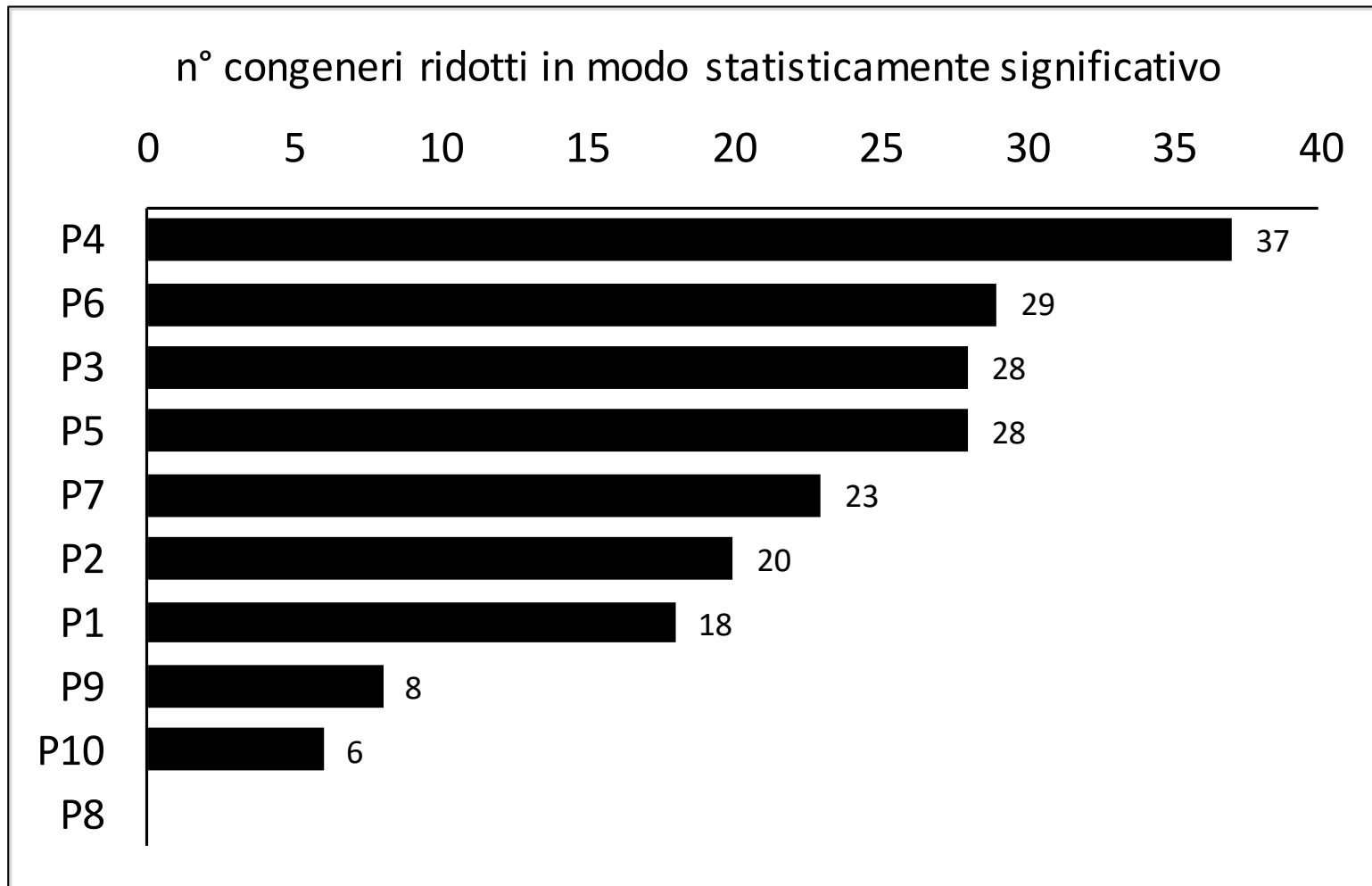
Risultati PCBs: famiglie di congeneri

- **MONO, DI:** non ridotti in modo statisticamente significativo
- **TRI-TETRA-PENTA:** P4, P6 and P7 → **20%-30%**
- **ESA – EPTA:** P4 and P6 → **20-25%**
- **OCTA:** non ridotti in modo statisticamente significativo
- **NONA-DECA:** P6 → **~15%**
- Generalmente gli esperimenti in serra della letteratura riportano delle **riduzioni più ottimistiche** in un **minor tempo**.

CONDIZIONI DI “SEMI-CAMPO”

SUOLO SIN BRESCIA-CAFFARO

Risultati PCBs: singoli congeneri (1)



Risultati PCDD/Fs: trattamenti migliori

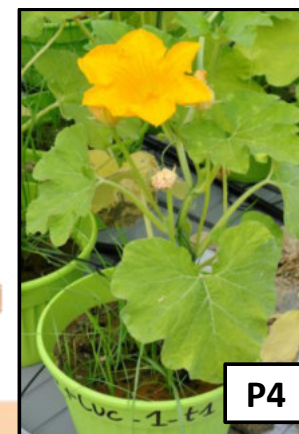
FESTUCA +
COMPOST



FESTUCA



FESTUCA +
ZUCCA



2+9

6+9

2+5

Numero
congeneri
PCDD + PCDF



Risultati PCDD/Fs: singoli congeneri

PCDDs

α : 0.05

α : 0.1

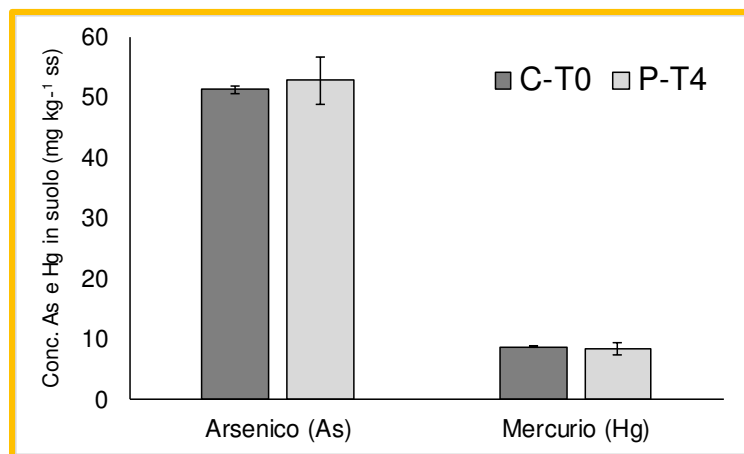
Riduzione % concentrazione PCDD: C-T0 vs P-T4							
	2378-TCDD	12378-PCDD	123478-HxCDD	123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
I-TEF	1	0.5	0.1	0.1	0.1	0.01	0.001
WHO98-TEF	1	1	0.1	0.1	0.1	0.01	0.0001
WHO05-TEF	1	1	0.1	0.1	0.1	0.01	0.0003
% in suolo	0.1	0.5	0.4	1	1	5	92
P3_FesNORM	s (24%)	s (16%)	s (21%)	s (17%)	s (12%)	s (15%)	ns (18%)
P4_Fes+Cuc	s (14%)	ns (5%)	s (21%)	ns (1%)	ns (+2%)	ns (6%)	ns (17%)
P6_FesCOM	ns (-21%)	s (13%)	s (30%)	ns (21%)	ns (14%)	ns (24%)	ns (10%)

PCDFs

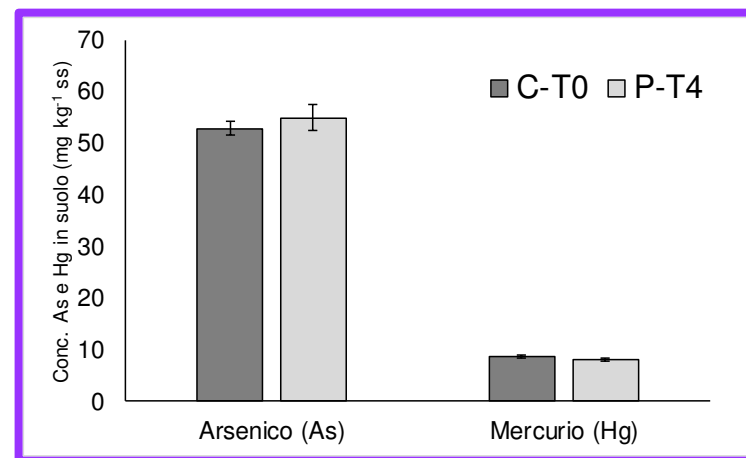
Riduzione % concentrazione PCDF: C-T0 vs P-T4										
	2378-TCDF	12378-PCDF	23478-PCDF	123478-HxCDF	123678-HxCDF	234678-HxCDF	123789-HxCDF	1234678-HpCDF	1234789-HpCDF	OCDF
I-TEF	0.1	0.05	0.5	0.1	0.1	0.1	0.1	0.01	0.01	0.001
WHO98-TEF	0.1	0.05	0.5	0.1	0.1	0.1	0.1	0.01	0.01	0.0001
WHO05-TEF	0.1	0.03	0.3	0.1	0.1	0.1	0.1	0.01	0.01	0.0003
% in suolo	17	9	6	14	3	2	4	8	4	33
P3_FesNORM	ns (11%)	s (19%)	s (16%)	s (15%)	s (16%)	s (15%)	s (14%)	s (12%)	s (13%)	s (23%)
P4_Fes+Cuc	ns (9%)	s (12%)	ns (12%)	s (11%)	s (10%)	s (10%)	s (10%)	ns (7%)	ns (7%)	ns (6%)
P6_FesCOM	ns (5%)	s (10%)	s (10%)	s (14%)	s (15%)	s (14%)	s (14%)	s (16%)	s (16%)	s (12%)

Risultati As e Hg

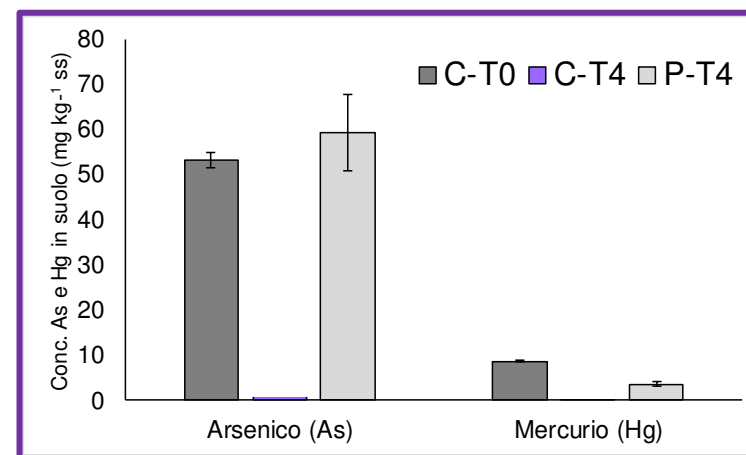
P8 – FELCE + SALICE



P9 – SENAPE INDIANA



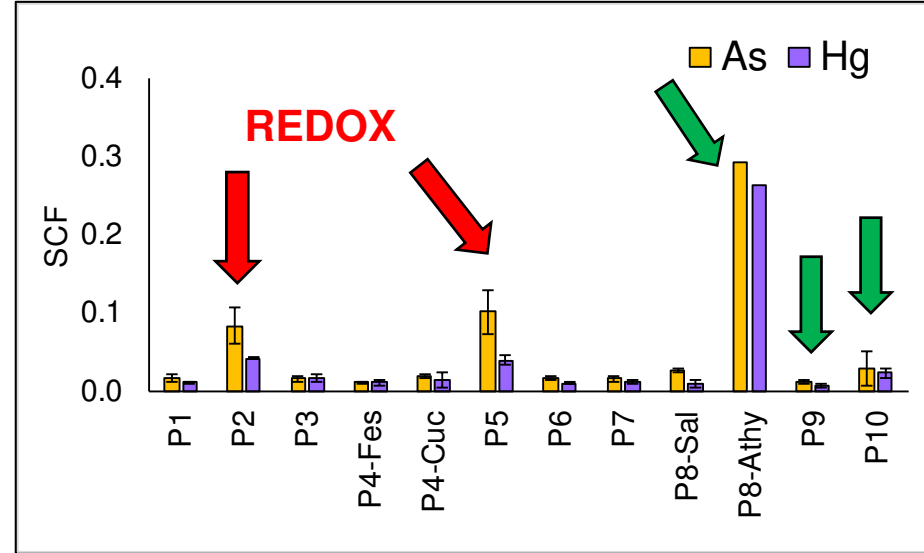
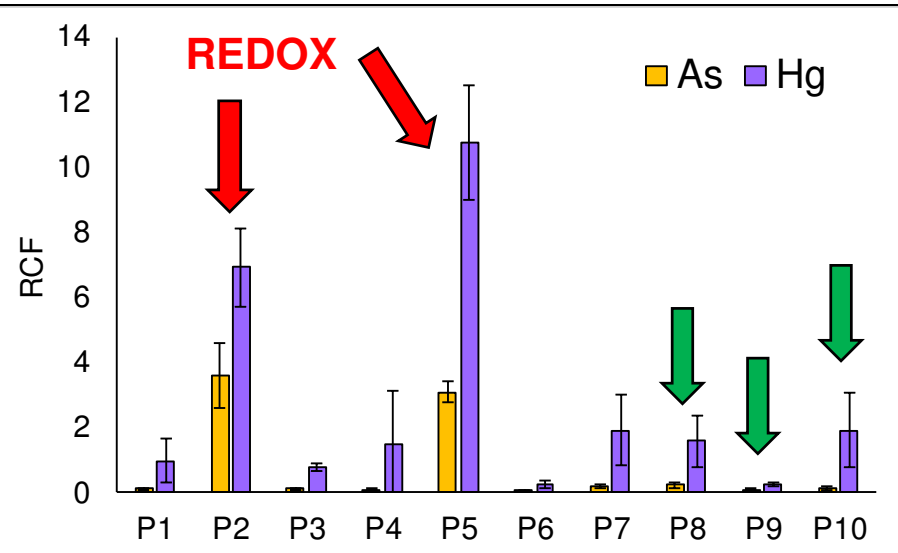
P10 – SENAPE INDIANA + TIO



Risultati As e Hg

RCF

SCF



$$RCF = \frac{Conc. radici \left(\frac{mg}{kg} \right)}{Conc. suolo \left(\frac{mg}{kg} \right)}$$

$$SCF = \frac{Conc. fusto/foglie \left(\frac{mg}{kg} \right)}{Conc. suolo \left(\frac{mg}{kg} \right)}$$

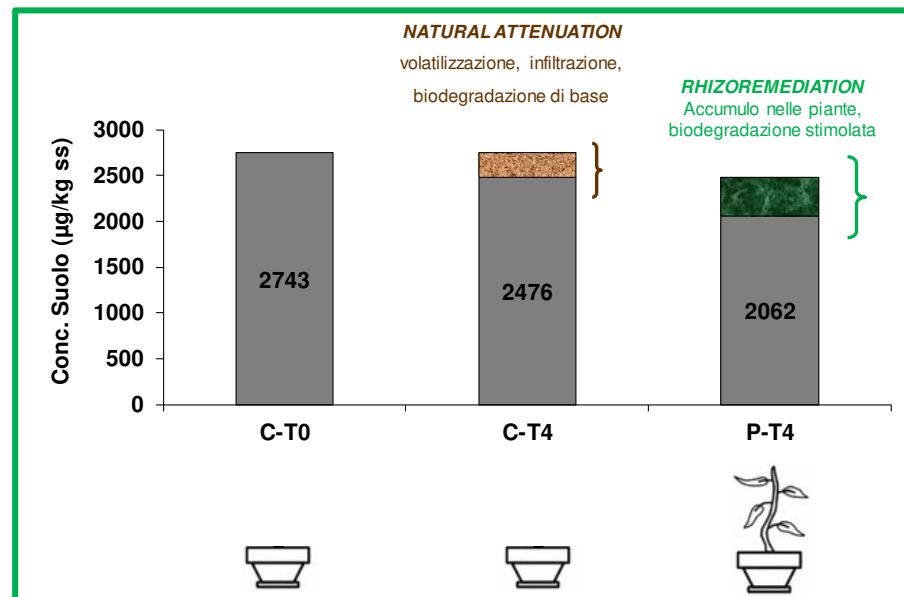
Calcolo emivite di *rhizoremediation*

CINETICA DEL 1° ORDINE

$$k_D = \left[\frac{-\ln(P_{T4} - C_{T0})}{t} \right]$$

$$HL_{rhizo} = \frac{0.693}{k_D}$$

dove k_D è il rate di degradazione (giorni^{-1}), $\ln$ è il logaritmo naturale, P_{T4} è la concentrazione finale nel trattamento ($\mu\text{g/kg ss}$), C_{T0} è la concentrazione iniziale nel controllo ($\mu\text{g/kg ss}$), t è il tempo (giorni) che intercorre tra $T0$ e $T4$ e HL_{rhizo} (half-life) è l'emivita di *rhizoremediation* della sostanza (giorni).



Emivite di *rhizoremediation*

Trattamenti più efficienti: P4 e P7



FESTUCA + ZUCCA



ERBA MEDICA

Emivite RR < Emivite NA

	Emivite NA	Emivite RR
PCB	1-23 anni	3-11 anni
PCDD	18-79 anni	4-8 anni
PCDF	8-23 anni	4-11 anni

NA: *natural attenuation* ^(a,b)

RR: *rhizoremediation*

^{a)} Paasivirta and Sinkkonen, 2009. *J. Chem. Eng. Data* 54, 1189–1213

^{b)} Sinkkonen and Paasivirta, 2000. *Chemosphere* 40, 943–949

Quali sono i tempi di risanamento per aree agricole selezionate se consideriamo emivite NA e se consideriamo RR?

Distribuzione spaziale dei contaminanti nel suolo e stima dei tempi di risanamento delle aree agricole -
Giuseppe Raspa (La Sapienza – Università di Roma).



Conclusioni

- ❖ Sono stati identificati 4 trattamenti (3 specie) che possono essere utilizzati per la bioremediation delle aree agricole del SIN Brescia-Caffaro.
- ❖ Le emivite di rhizoremediation sono più basse di quelle di natural attenuation.
- ❖ L'esperimento in serra è stato condotto a un singolo livello di concentrazione mentre i dati di concentrazione di campo mostrano un'ampia variabilità di concentrazioni (fino a 2 ordini di grandezza).
- ❖ Occorre verificare l'efficacia della rhizoremediation in presenza di suoli che presentano un ampio range di concentrazioni di contaminanti.
- ❖ I risultati mostrano l'effettiva fattibilità delle azioni di rhizoremediation adottabili nel SIN Brescia-Caffaro sia per PCB che PCDD e PCDF.
- ❖ Occorre invece ottimizzare le condizioni sperimentali per As e Hg.



SAPIENZA
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COMUNE DI BRESCIA

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- Università degli Studi di Milano
- Sapienza - Università di Roma
- ERSAF
- Comune di Brescia

GRAZIE PER L'ATTENZIONE!