Phytoremediation of contaminated soils: facts and challenges

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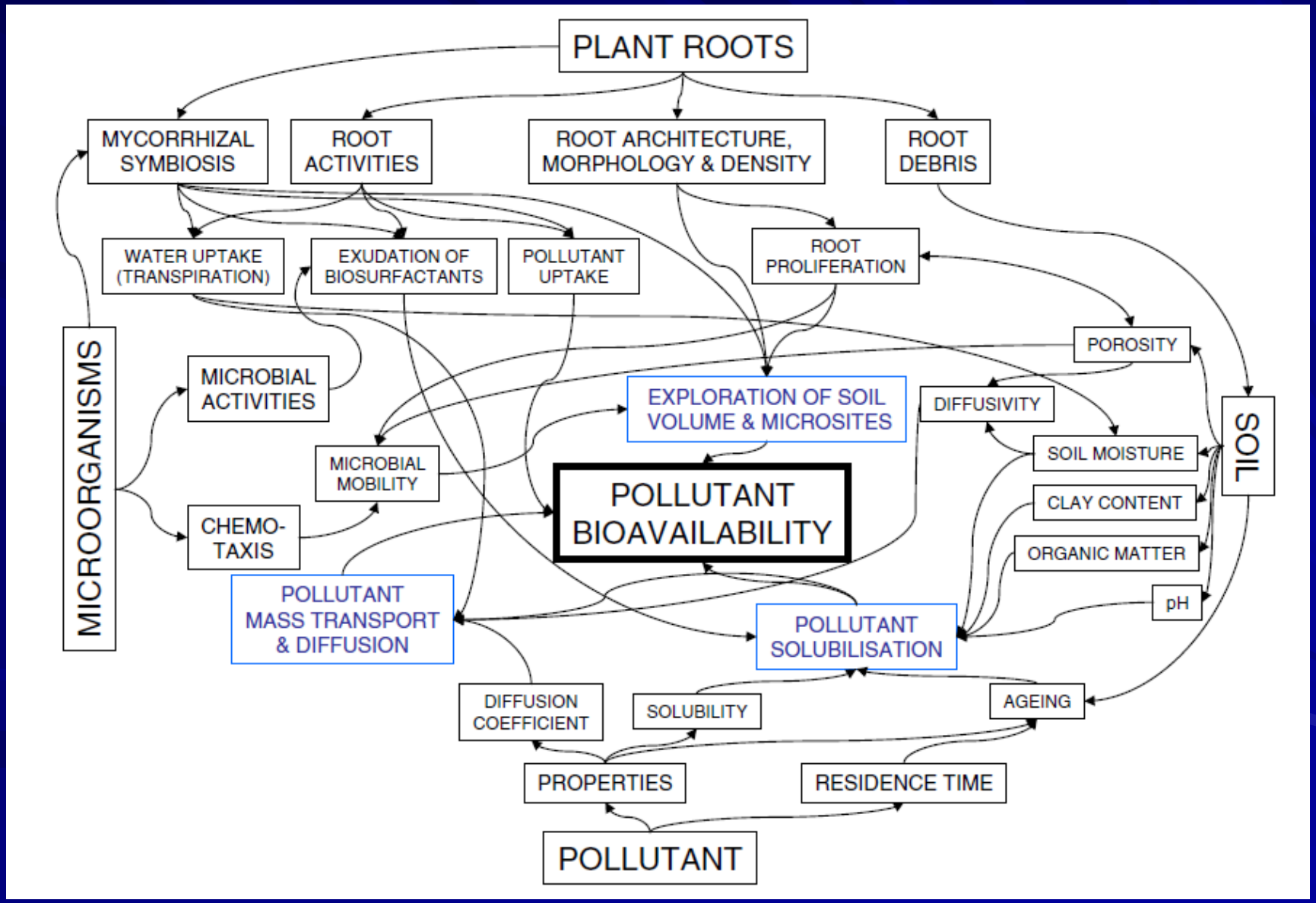
ETH, Zurich, December 2016

Complex interactions in contaminated soils

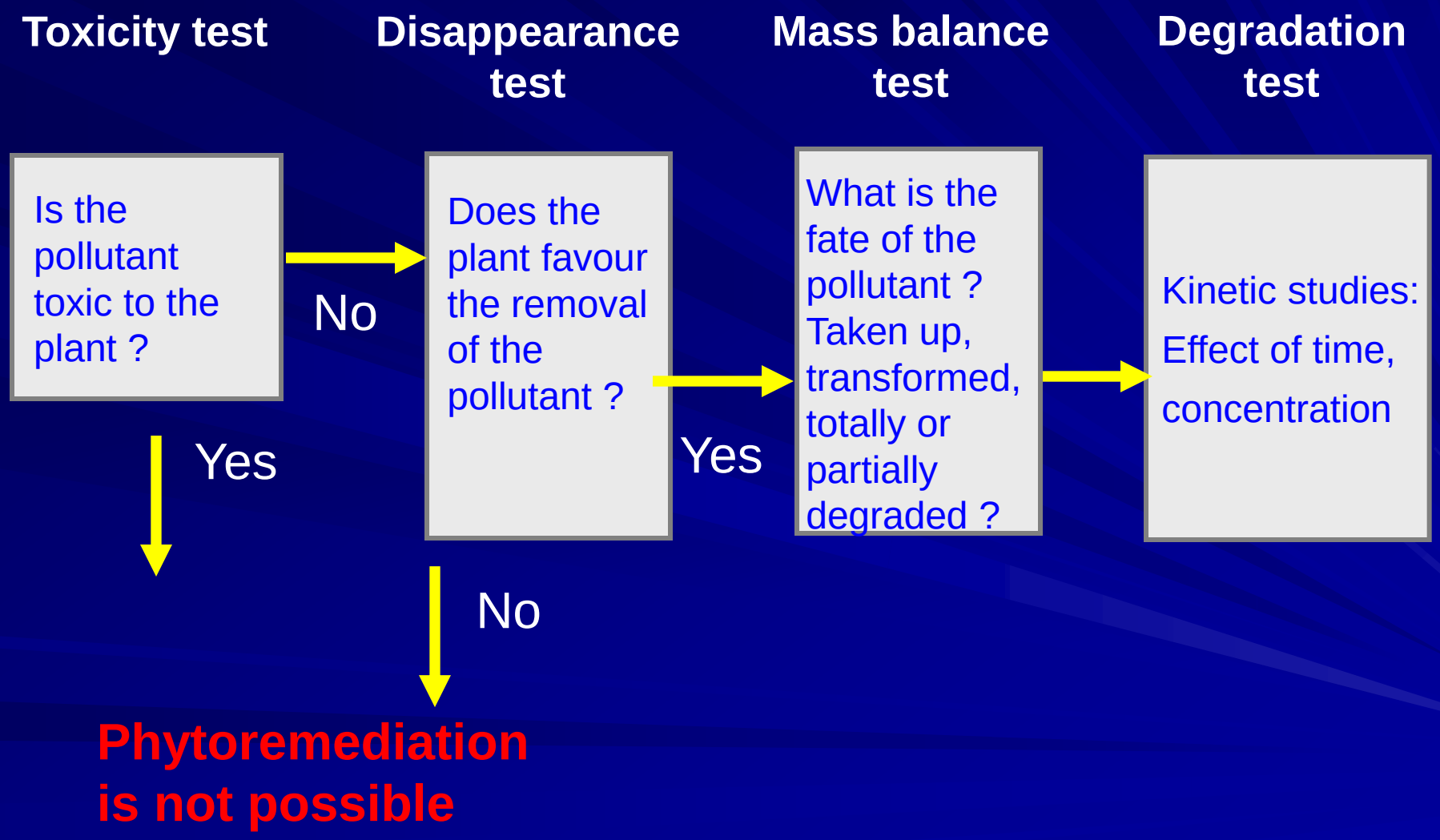


Wenzel (2009) Plant Soil 321:385-408

It is ecologicallysafe and eonomicallyviable

Risk assessment of phytoremediation technique

Tests to be done before any phytoremediation trial



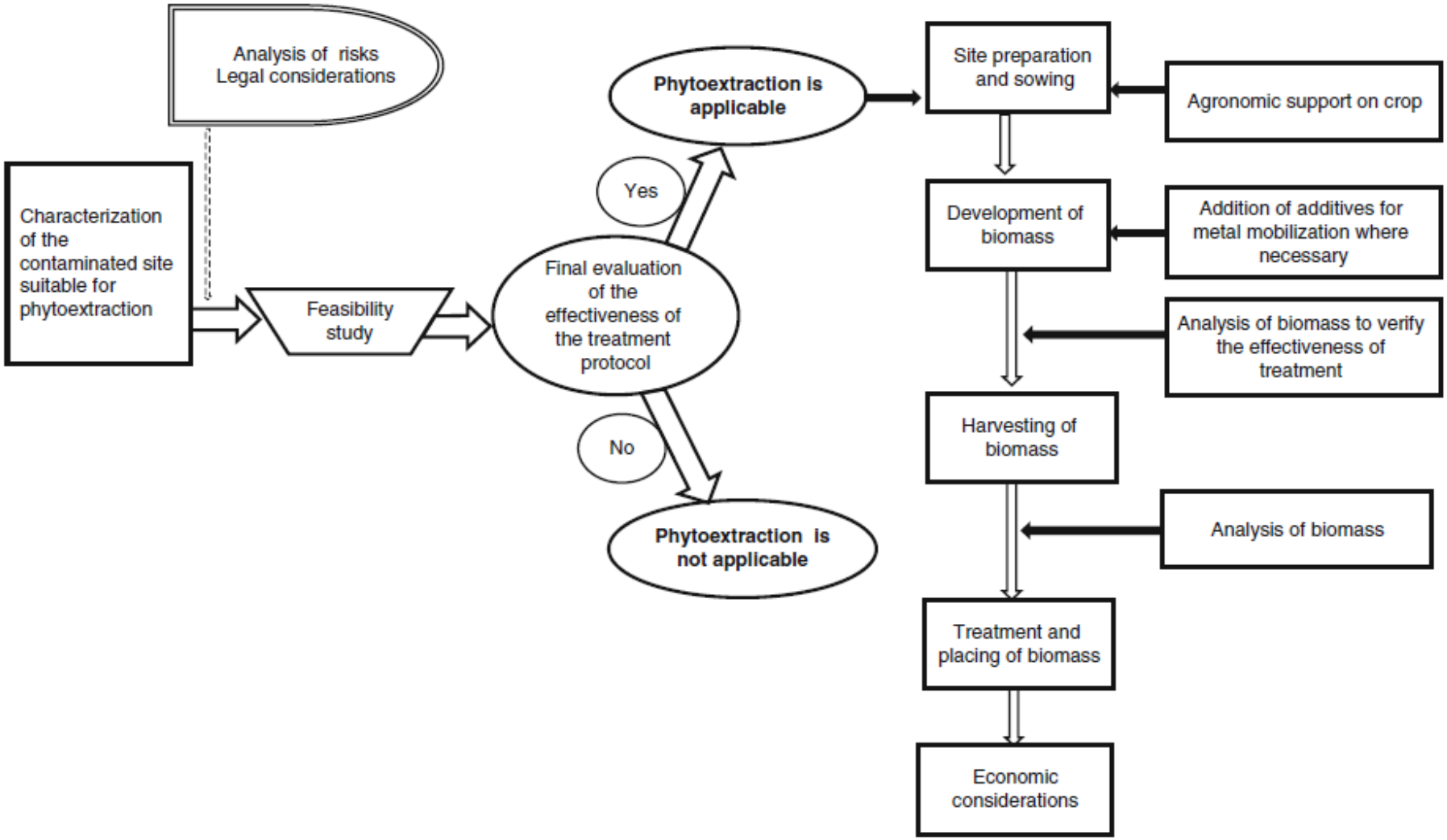


Fig. 2.1 Diagram of applicability of the phytoextraction procedure in situ

Barbafieri et al. (2013) Chapter 2 in D.K Gupta (ed.) Plant-Based Remediation Processes

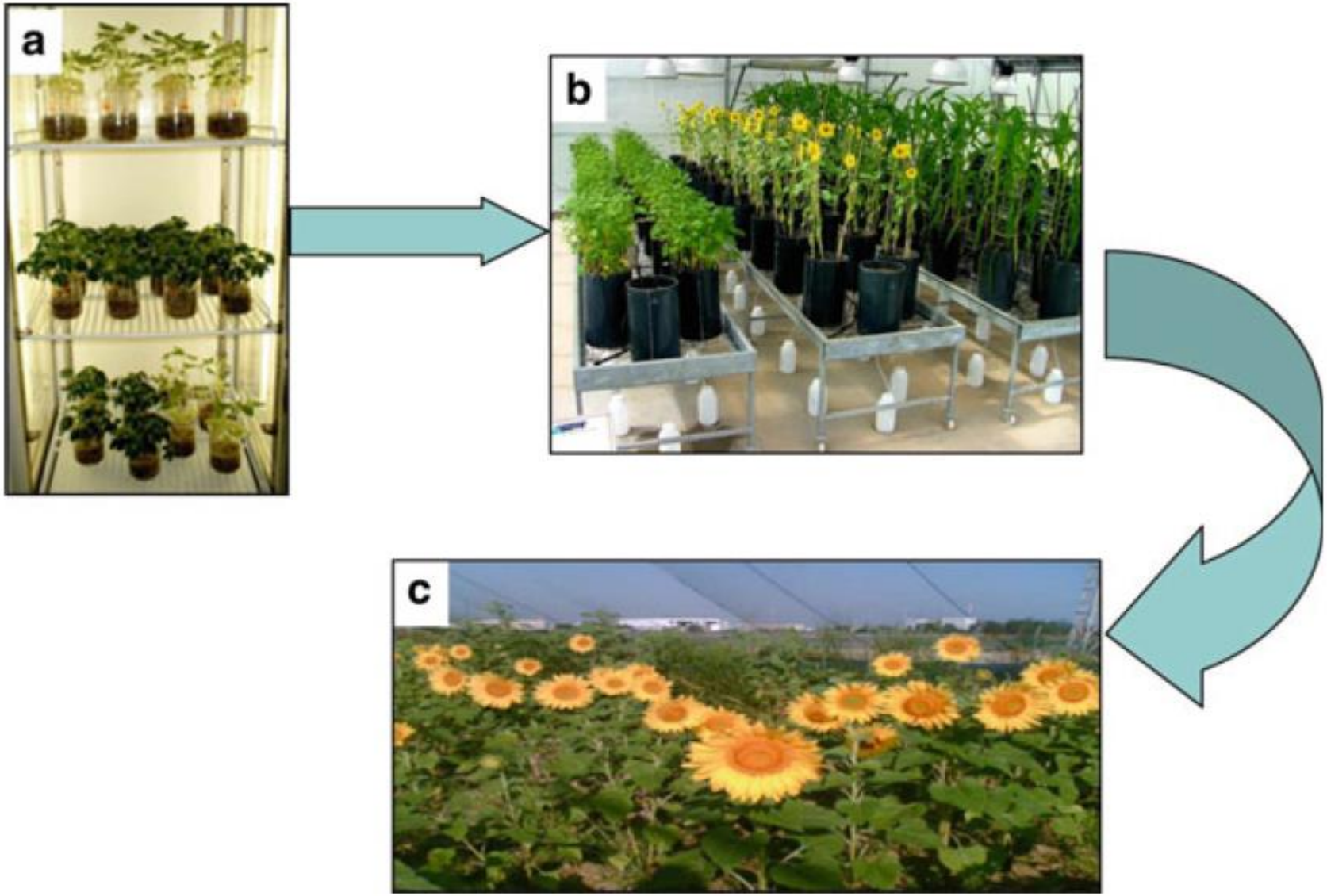


Fig. 2.3 Site-specific feasibility test: (**a**) microcosm, (**b**) mesocosm, (**c**) field test

Barbafieri et al. (2013) Chapter 2 in D.K Gupta (ed.) Plant-Based Remediation Processes

1) Trace elements

A. Mahar et al. / Ecotoxicology and Environmental Safety 126 (2016) 111–121

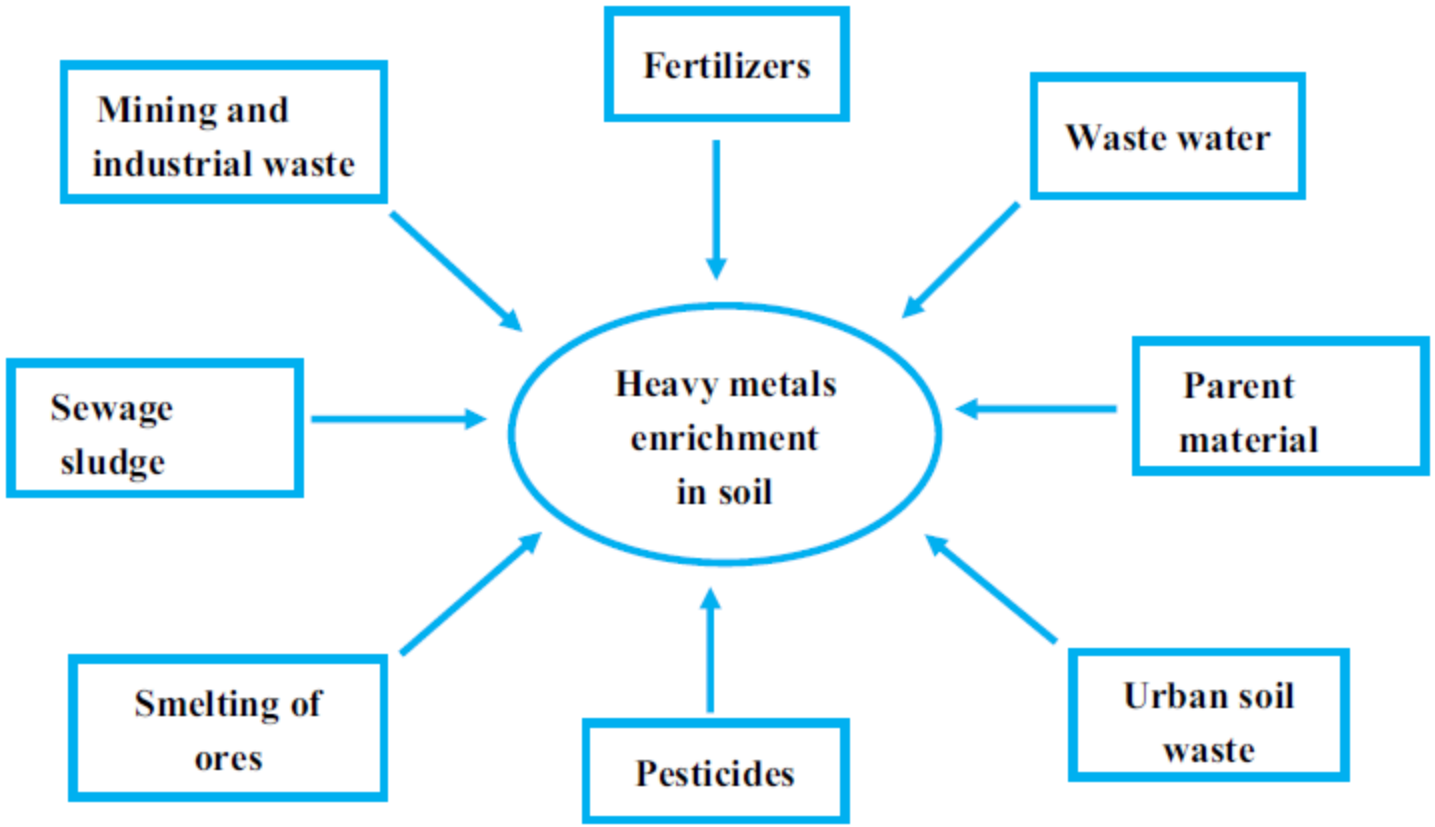


Fig. 1. Major sources of heavy metals in soil.

L. Rocha et al. / Chemosphere 84 (2011) 1495–1505

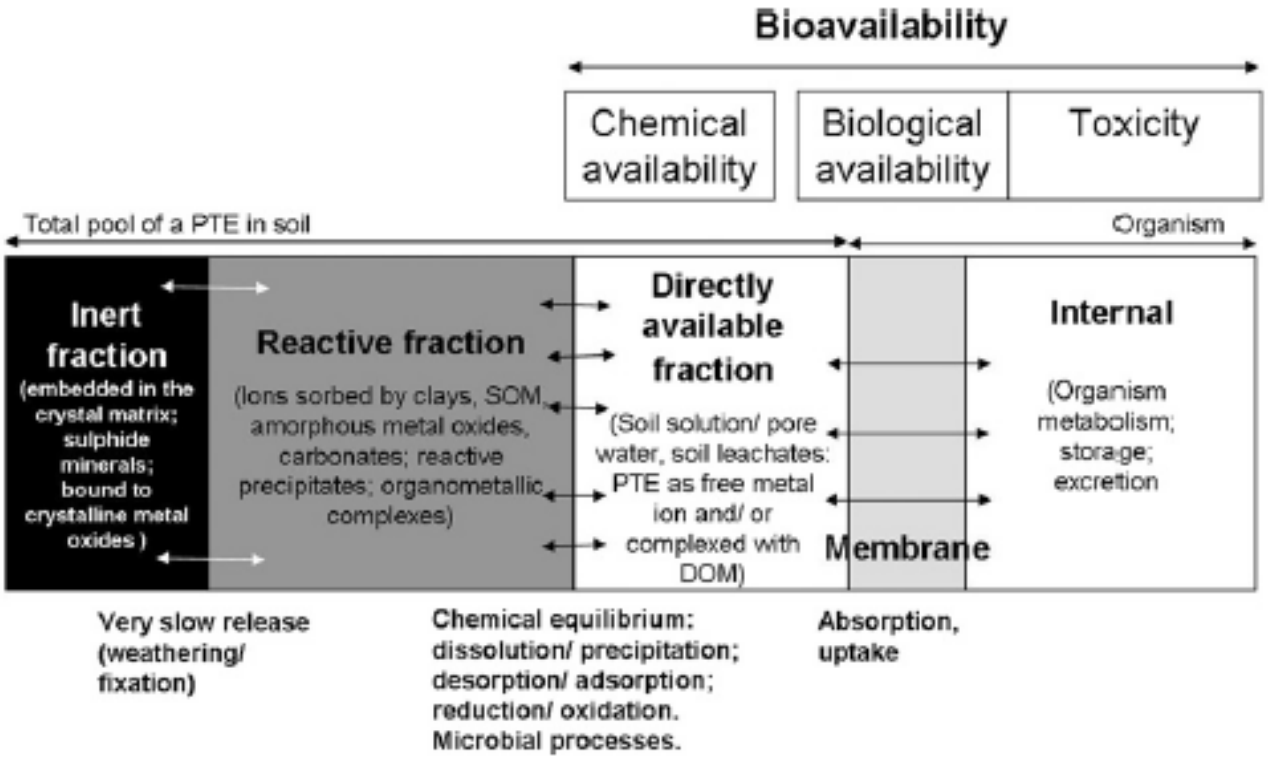
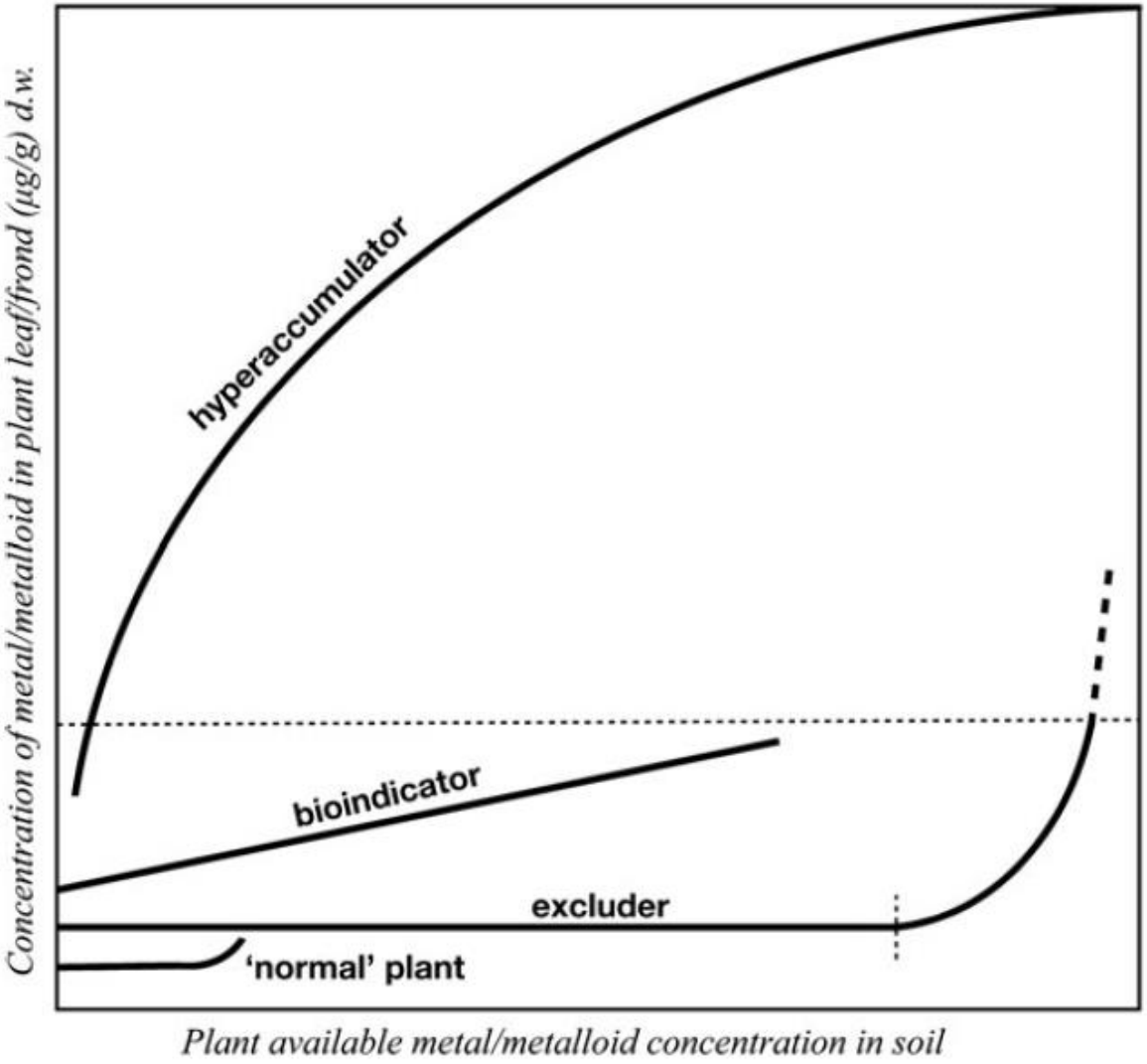
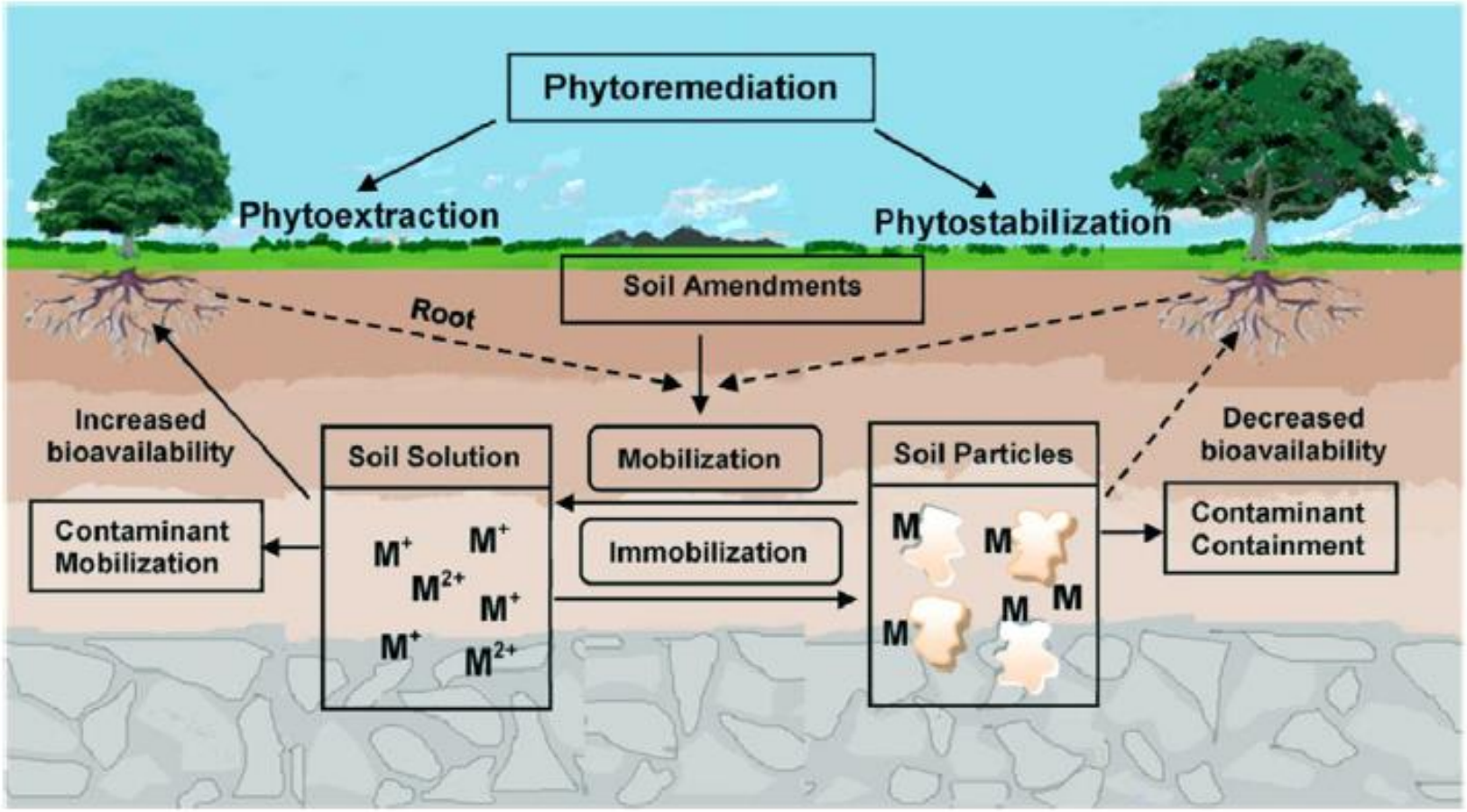


Fig. 1. Overview of processes controlling the bioavailability of PTE's in soils to organisms (adapted from Loibner et al., 2006 and Römkens et al., 2009).



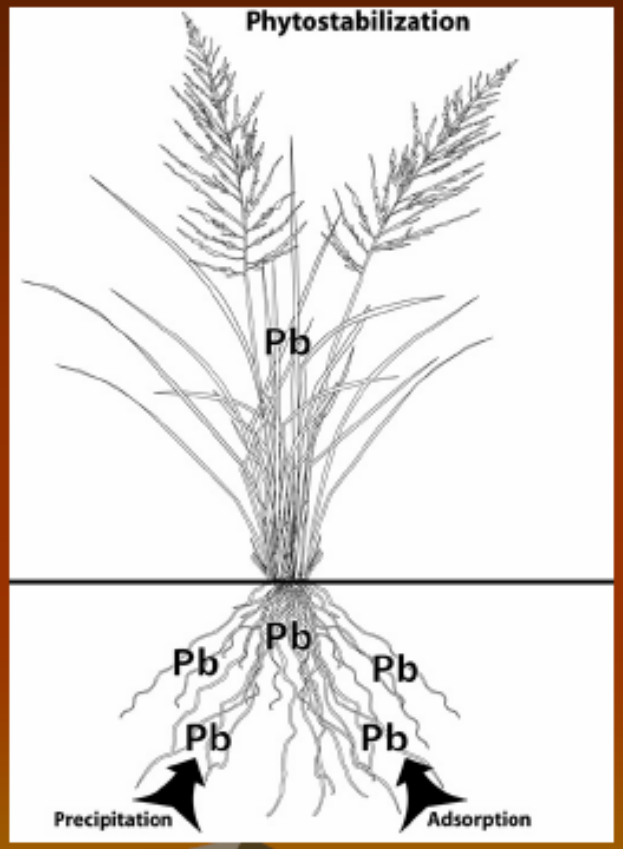
Van der Ent et al.

A. Mahar et al. / Ecotoxicology and Environmental Safety 126 (2016) 111–121



1.1) What is phytostabilization?

- Remediation technology uses **native** plants to:
  - **Immobilize contaminants in soil through adsorption and precipitation, rhizosphere**
  - **Use plant roots to prevent migration via:**
    - **Wind erosion**
    - **Water/soil erosion**
    - **Leaching**
    - **Safe to wildlife**
- EPA, Introduction to Phytoremediation
  EPA/600/R-99/107



Lysimeter experiment of phytostabilisation (coal fly ash+peat) of Cu (250 mg kg^{-1} DW) and Pb (2560 mg kg^{-1} DW) contaminated sandy soil after one year (Kumpiene et al. 2007)

Table 3
Leaching of Cu and Pb from the untreated and treated soils as assessed by the batch test according to the 2^2 factorial design (±SD, $n = 3$)

	Leachate		Cu		Pb	
	pH	EC		Efficiency		Efficiency
		μS cm^{-1}	mg kg^{-1}	%	mg kg^{-1}	%
Untreated soil	4.1	75	26.6 ± 0.2		152.0 ± 1.5	
Soil + organic matter (OM)	3.9	145	6.8 ± 0.1	74.5	59.3 ± 0.6	61.0
Soil + coal fly ash (CFA)	11.6	1488	2.4 ± 0.0	91.1	19.6 ± 0.0	87.1
Soil + OM + CFA	7.5	541	0.5 ± 0.0	98.2	0.2 ± 0.0	99.9

OM = 5 kg DW peat / 100 kg DW soil
CFA = 5 kg coal fly ash / 100 kg DW soil

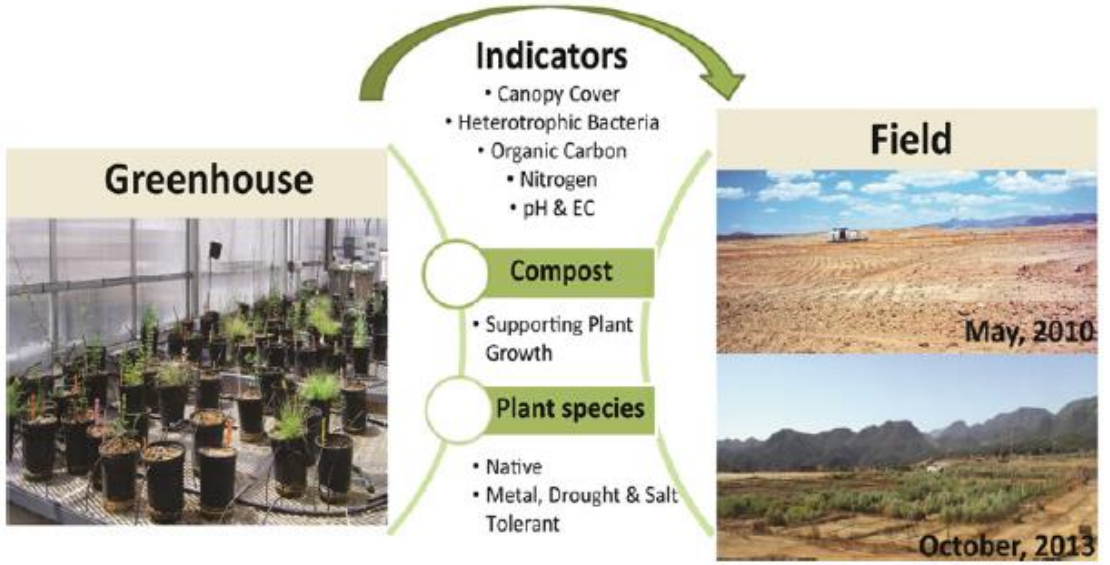
Table 5
Cu and Pb concentrations in plant shoots, mg kg^{-1} DW (±SD, $n = 3$)

	2003				2004			
	U	U + V	T	T + V	U	U + V	T	T + V
Cu	NV	47.1 ± 0.9	NV	13.5 ± 0.7	NV	NV	2.0 ± 0.1	1.3 ± 0.0
Pb	NV	221.5 ± 10.7	NV	13.8 ± 0.7	NV	NV	17.3 ± 1.1	12.8 ± 0.9

NV, no vegetation. Soil: U, untreated; U + V, untreated with sowed grass; T, treated; T + V, treated with sowed grass.

Agrostis capillaris; Anthoxanthum odoratum; Bromus hordeaceus; Deschampsia flexuosa; Festuca rubra; Festuca ovina

- Cap and plant is the current costly standard for mine tailings reclamation
- We assessed direct planting for remediation of acidic metalliferous mine tailings
- 60-day greenhouse pot studies translated successfully to this 41-month field trial
- A single compost application supported plant establishment and soil development
- Direct planting with compost is a viable alternative for treatment of mine tailings



Gil-Loaiza et al (2016) Science of the Total Environment 565:451-461

Fig. 1. (A) An aerial view of the IKMHSS tailings. The black square delineates the study area. The inset shows the location of the IKMHSS site in Yavapai County, AZ. (B) a map of the 24 plots (9.6 m × 15 m per plot) showing the location of all treatments and replicates. Numbers indicate treatment: (1) unamended control; (2) 10% compost – seeded with buffalo grass (BG) and mesquite (MQ); (3) 15% compost – unseeded; (4) 20% compost – unseeded; (5) 15% compost – seeded; (6) 20% compost –seeded.

Table 5
Plant composition based at the IKMHSS field site from 2010 to 2013.

Treatments	Plant type	Plant composition (%)			
		2010	2011	2012	2013
20% C; Seeded	Grasses[a]	13.8 (4.1)	13.6 (3.7)	23.6 (11.9)	24.8 (16)
	Shrubs[b]	86.1 (4.0)	83.4 (3.2)	67.9 (14.6)	56.4 (12)
	Trees[c]	0.0	0.0	0.0	0.0
	Weeds[d]	0.0	2.9 (4.4)	8.5 (8.9)	18.8 (14)
20% C; Unseeded	Grasses	0.0	0.0	0.0	0.0
	Shrubs	14.7 (26.4)	26.5 (49)	18.0 (22.5)	19.4 (16)
	Trees	0.0	0.0	0.0	0.0
	Weeds	85.3 (26.4)	73.5 (49)	82.0 (25.4)	80.6 (16)

20% compost in top 15 cm of soil = 456 t ha^{-1}

Seeding rates of 6 native desert plants

Grasses: 90 kg ha^{-1} *Buchloe dactyloides*; 56 kg ha^{-1} *Festuca arizonica*
Shrubs: 56 kg ha^{-1} *Atriplex lentiformis*; 11 kg ha^{-1} *Cercocarpus montanus*
Trees: 0.15 kg ha^{-1} *Prosopis juliflora*; 1 kg ha^{-1} *Acacia greggi*

Gil-Loaiza et al (2016) Science of the Total Environment 565:451-461

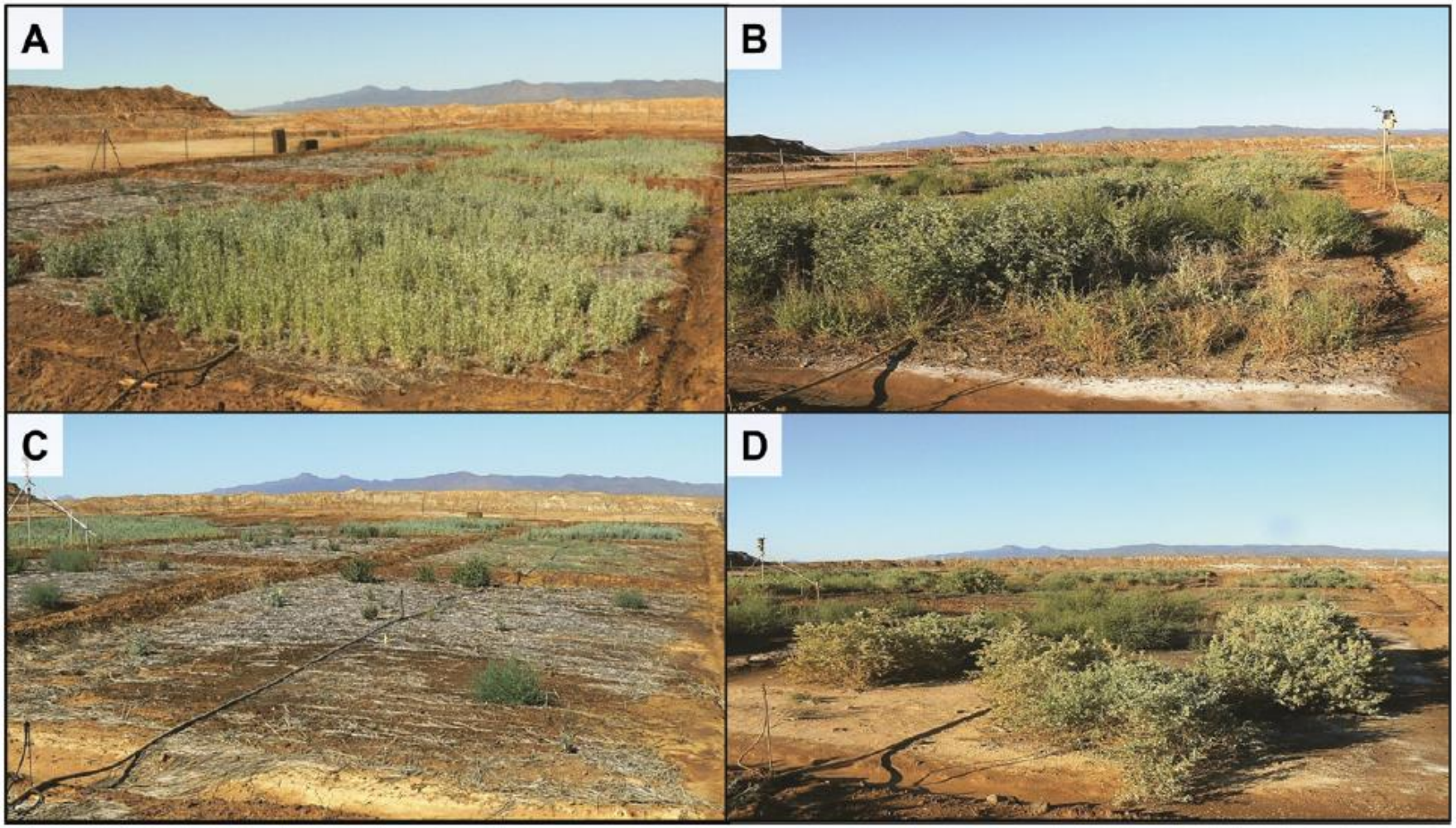
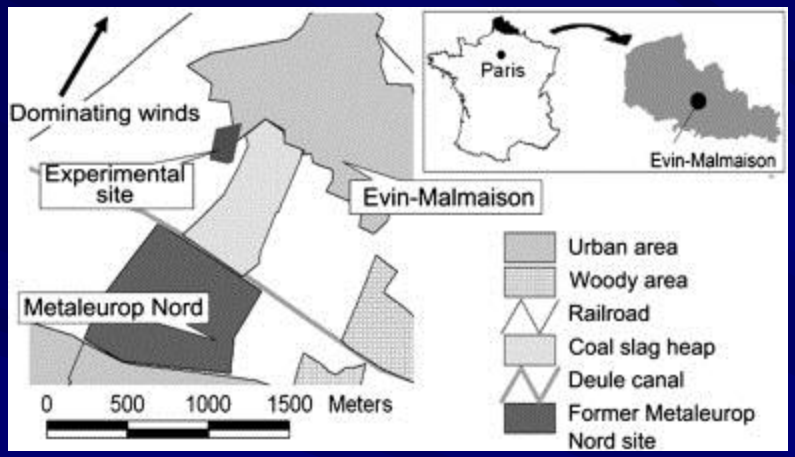
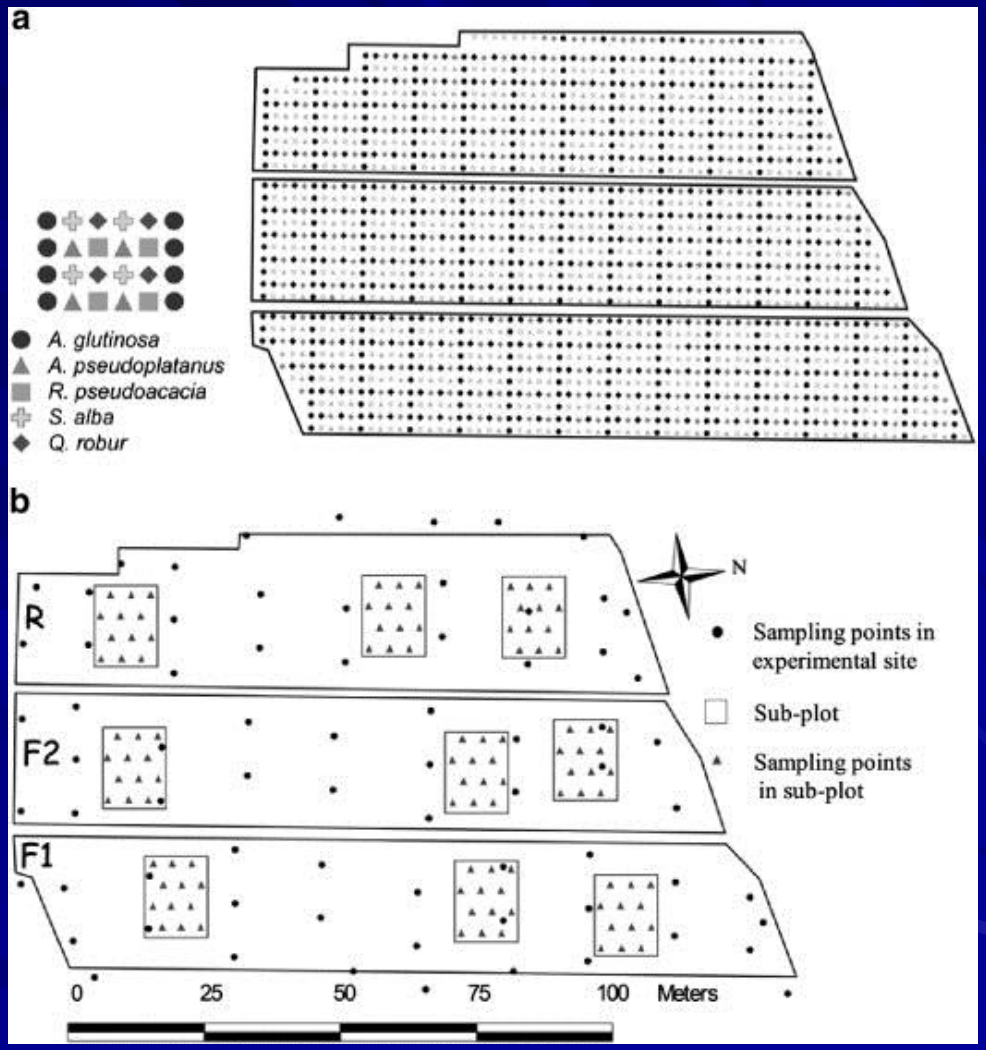


Fig. 3. A comparison of quailbush plants in the 20% compost seeded treatment (A) 2010; (B) 2012 and the 20% compost unseeded treatment in (C) 2010; (D) 2012.

Gil-Loaiza et al (2016) Science of the Total Environment 565:451-461

Addition of fly ash for phytostabilisation of soils highly contaminated with Pb, Cd and Zn (Northern France)





3 plots of about 3000 m² each:
R: not amended (control)
F1, F2: amended with fly ash (23 kg/m²)

In 2000, planted with 1800 trees mix:
Alnus glutinosa (black alder)
Acer pseudoplatanus (sycamore maple)
Robinia pseudoacacia (black locust)
Salix alba (white willow)
Quercus robur (pedunculate oak)
+ Herbaceous mixture cover

Lopareva-Pohu et al. (2011) Sci Tot Environ 409:647-654; Environ Pollut 159: 1721-1729

Table 2
Total Cd, Pb and Zn concentrations in the experimental site top soils in 2001 and 2008.

	R	F1	F2	R	F1	F2
	2001			2008		
Cd (mg kg^{-1})	16.5^{a} ± 0.6	17.8^{a} ± 0.4	16.7^{a} ± 0.6	16.6^{a} ± 0.6	18.0^{a} ± 0.3	17.8^{a} ± 0.5
Pb (mg kg^{-1})	939^{ab} ± 32	1023^{a} ± 24	929^{b} ± 37	926^{a} ± 29	967^{a} ± 16	900^{a} ± 30
Zn (mg kg^{-1})	1155^{a} ± 40	1256^{a} ± 28	1178^{a} ± 41	1135^{a} ± 42	1211^{a} ± 26	1127^{a} ± 43

Data are means ± SE; R: n = 24, F1: n = 27, F2: n = 21; a and b denote significant differences between plots; analysis was carried out separately for each year (Kruskal–Wallis test, $p < 0.05$).

Table 2
Cd, Pb and Zn concentrations in *T. repens* and *L. perenne* roots and shoots (mg kg^{-1} DW) (R: non amended-contaminated soil, F1 and F2: Coal fly ash amended soils).

Soil	Cd	Pb	Zn	Cd	Pb	Zn
	Roots			Shoots		
	T. repens					
R	139 ± 11.0 ^{a}	204 ± 12.3 ^{a}	1070 ± 88.0 ^{a}	8.2 ± 0.3 ^{a}	19.6 ± 2.1 ^{a}	166 ± 3.6 ^{a}
F1	75.1 ± 8.0 ^{b}	140 ± 22.6 ^{b}	424 ± 45.9 ^{b}	2.1 ± 0.1 ^{b}	9.3 ± 1.1 ^{b}	53.3 ± 2.1 ^{b}
F2	88.9 ± 8.4 ^{b}	108 ± 10.1 ^{b}	466 ± 29.0 ^{b}	1.6 ± 0.1 ^{b}	5.8 ± 1.2 ^{b}	61.9 ± 1.7 ^{b}
	L. perenne					
R	110 ± 4.4 ^{a}	470 ± 21.9 ^{ab}	1062 ± 43.5 ^{a}	3.4 ± 0.2 ^{a}	23.2 ± 1.0 ^{a}	172 ± 23.3 ^{a}
F1	88.7 ± 3.5 ^{b}	641 ± 59.8 ^{a}	754 ± 32.9 ^{b}	1.6 ± 0.1 ^{b}	12.2 ± 0.9 ^{b}	94.8 ± 7.6 ^{b}
F2	91.3 ± 1.9 ^{b}	419 ± 29.9 ^{b}	601 ± 22.7 ^{b}	1.7 ± 0.1 ^{b}	20.5 ± 2.2 ^{a}	89.6 ± 10.0 ^{b}

Data are means ± SE. a, b, c denote significant differences between treatments ($p < 0.05$, ANOVA Kruskal Wallis, $n = 5$).

Challenges for phytostabilisation

Phytostabilisation (aided)

Still questions to answer:

how permanent?

are there universal treatments?

comparisons under similar conditions

new combinations to enhance stabilization

full assessment of effects on range of properties and soil functions or induced deficiencies

1.2) Phytoextraction

Heavy metal concentrations (mg kg^{-1}) in shoots and roots of different species in the Dornach field experiment and outputs (g ha^{-1}) (Keller et al. 2003)
Concentrations in the first 20 cm of soil (mg kg^{-1}): 2.5 Cd, 550 Cu, 650 Zn

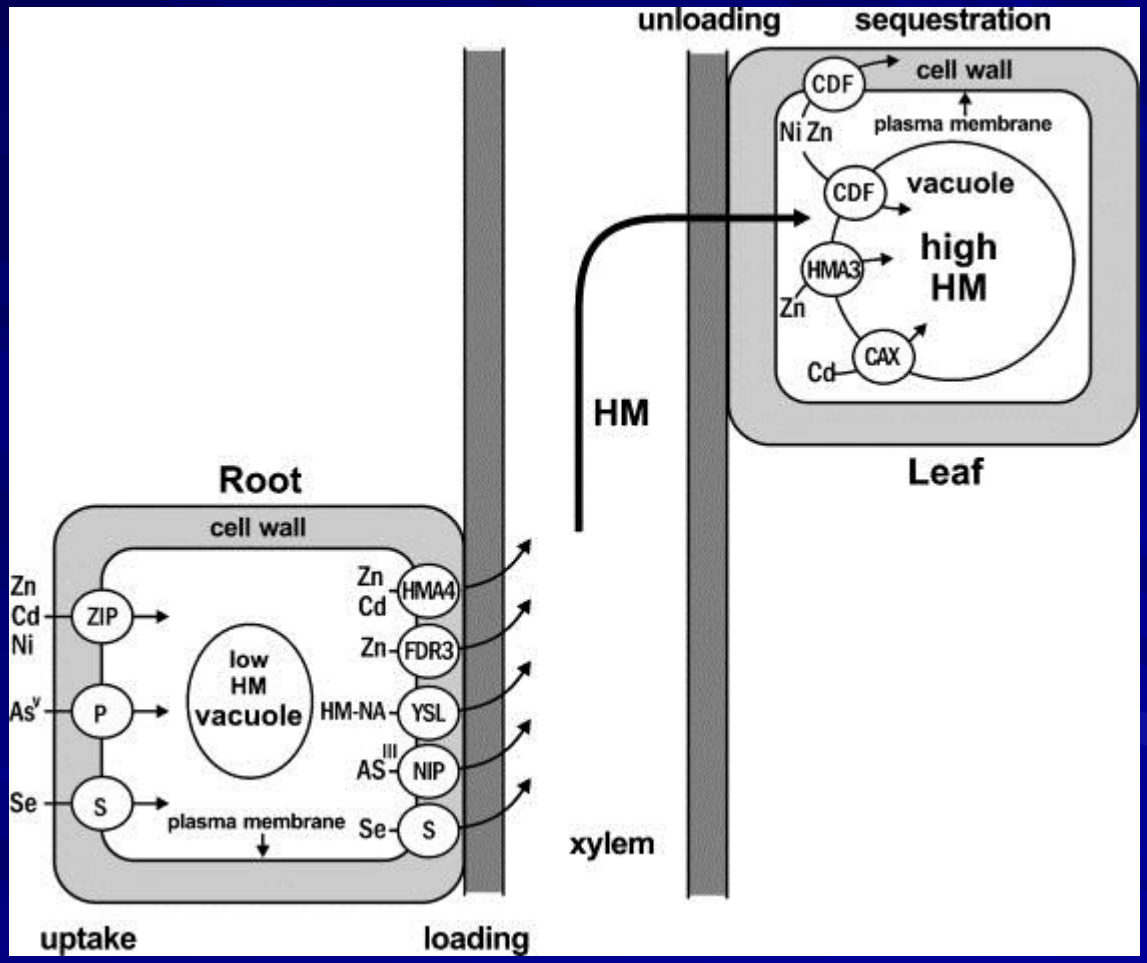
Plant	**Cd** shoot	**Cu** shoot	**Zn** shoot	**Cd** root	**Cu** root	**Zn** root	**Yield** t h^{-1}	**Cd** out	**Cu** out	**Zn** out
Thlaspi (Noccaea)	184	53	5265	168	71	693	0.9	**179**	50	**5052**
Indian mustard	1.0	20	124	0.3	32	45	7.3	6.95	146	894
Tobacco	3.5	38	146	0.6	26	36	12.6	41.7	**474**	1834
Maize	0.6	10	129	0.7	38	41	15.6	9	163	1998
Willow	3.4	14	294	nd	nd	nd	13.2	44	187	**3851**

T. caerulescens is a small, slow growing plant – Thus it's utility is as an interesting model system for understanding how plants accumulate and tolerate high levels of metals

The use of plants producing an important biomass with added value

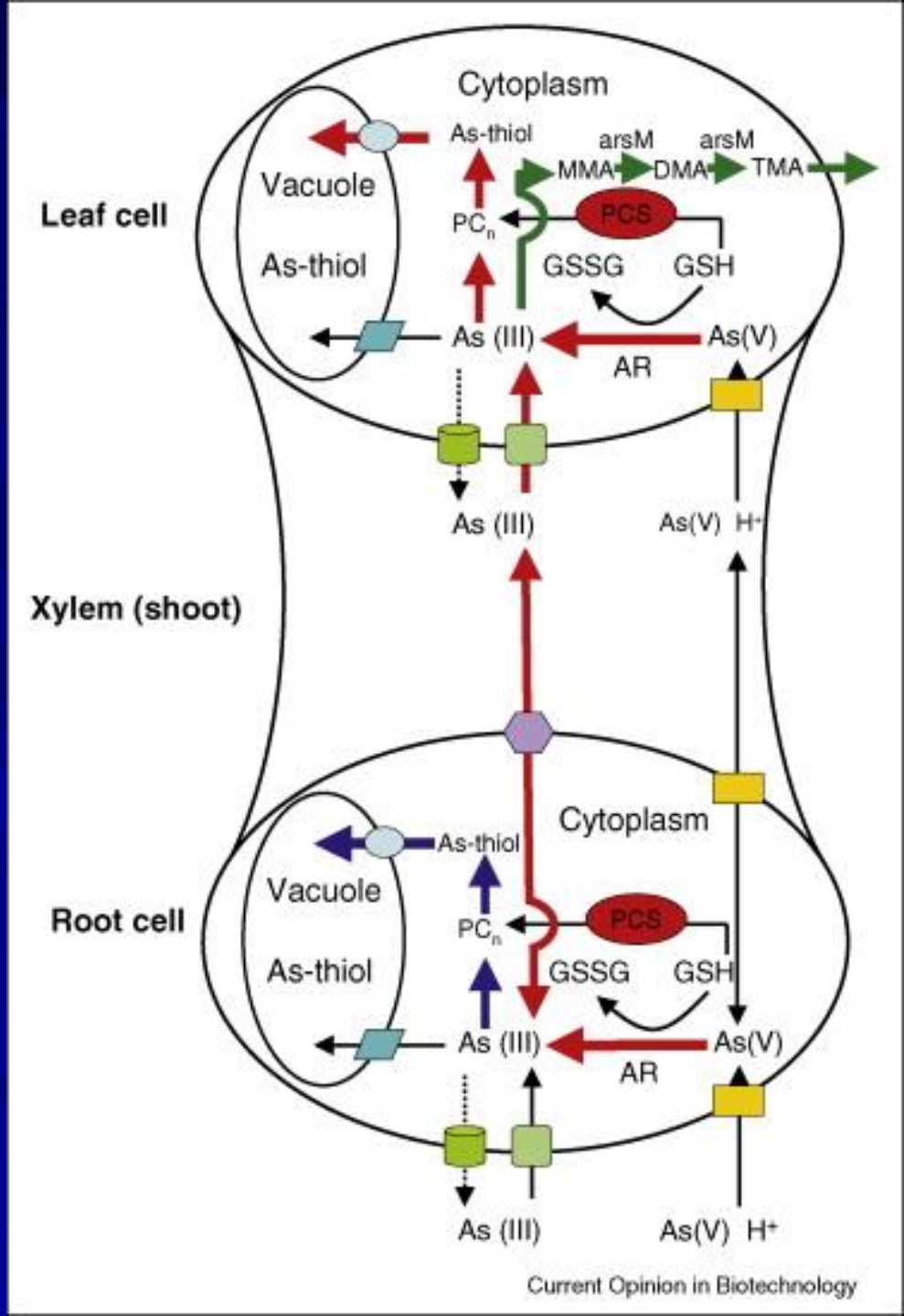
Short-rotation coppice willows

Transport systems involved in the uptake, root-to-shoot translocation and sequestration of hyperaccumulator plants



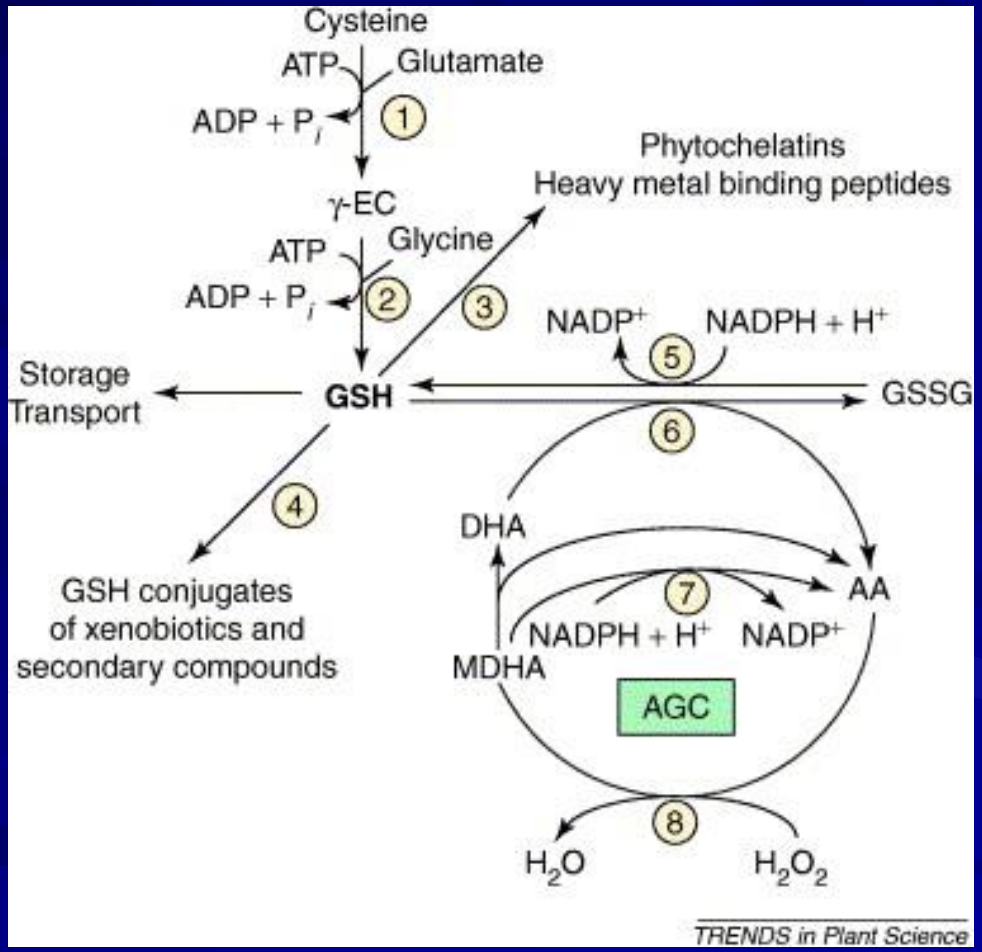
Rascio and Navari-Izzo (2011) Plant Sci 180:169

Arsenic uptake and metabolism in plants



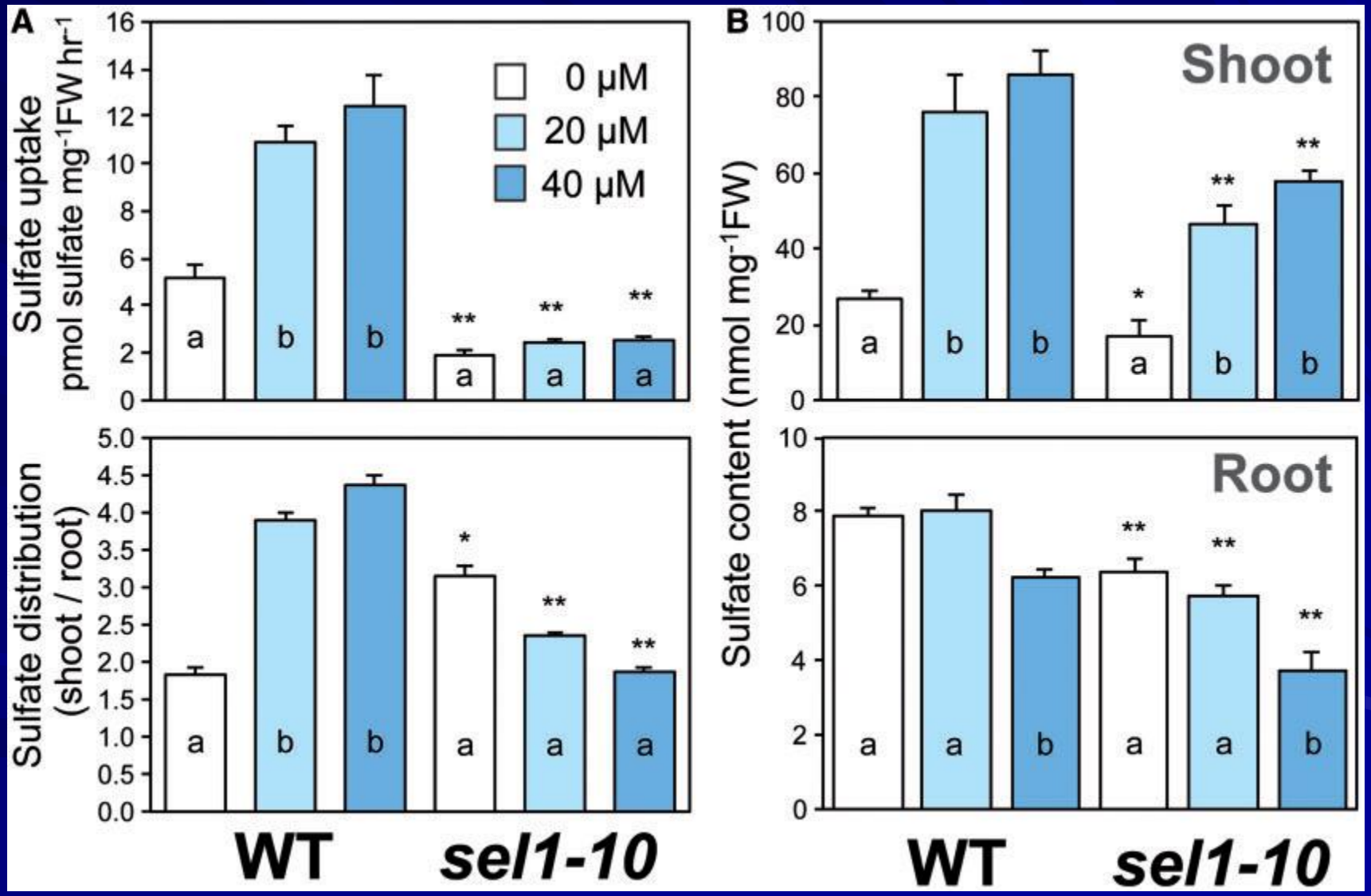
Zhu and Rosen (2009)
Current Opinion Biotechnol
20: 220-224

Biosynthesis and multifunctional role of glutathione (GSH) and phytochelatins



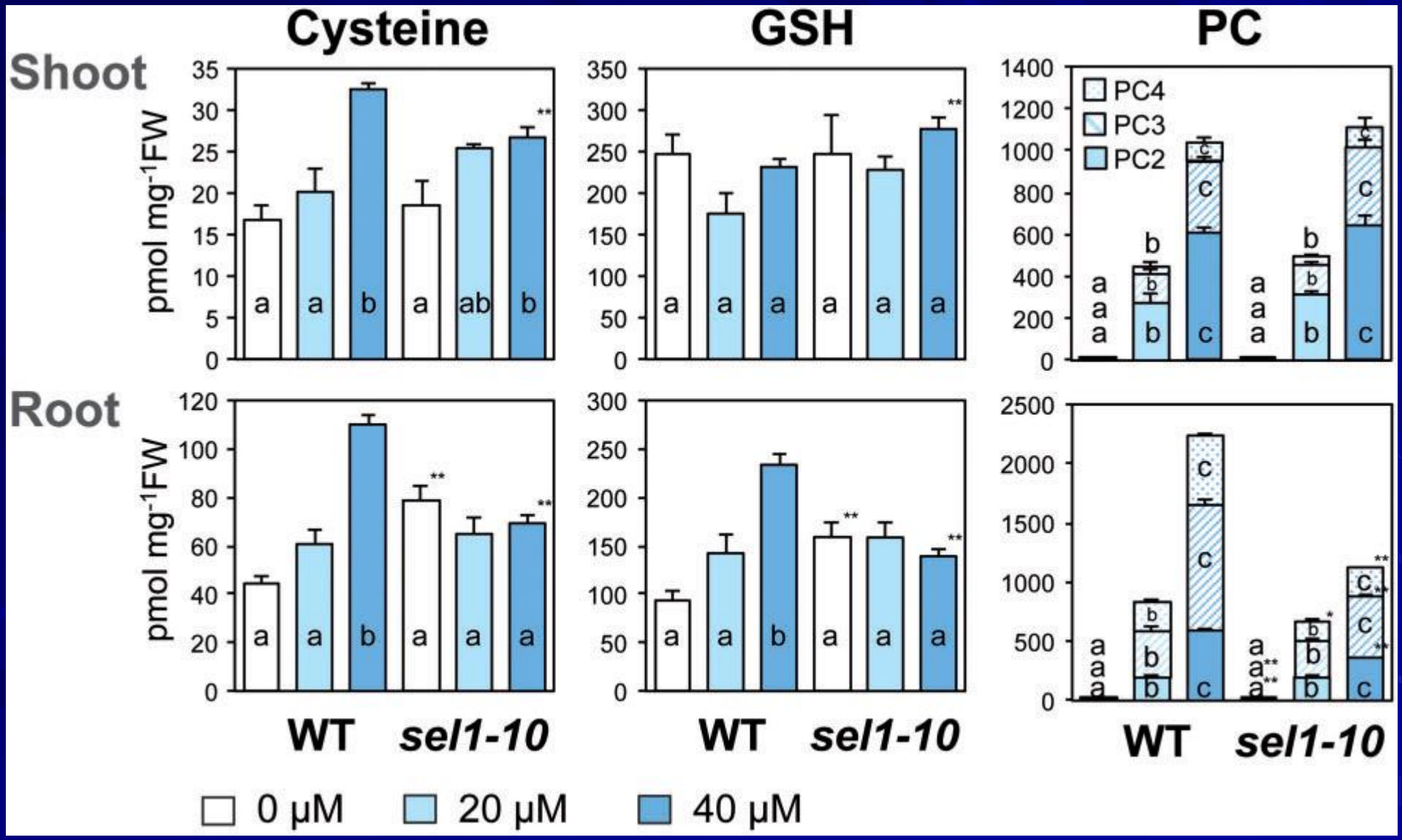
Rausch and Wachter (2005) Trends Plant Sci 10:503-509

Effect of Cd on the uptake, distribution and accumulation of sulfate in wild type and a mutant of *Arabidopsis thaliana*



Yamaguchi et al (2016) Plant Cell Physiol 57:2353-2366

Effect of Cd on cysteine, glutathione and phytochelatins in wild type and a mutant of *Arabidopsis thaliana*



Yamaguchi et al (2016) Plant Cell Physiol 57:2353-2366

Hyperaccumulation
(plants growing in their natural habitats)

100 mg/kg dried foliage: **Cd, Se, Tl**

300 mg/kg dried foliage : **Co, Cu, Cr**

1’000 mg/kg dried foliage : **Ni, Pb, As**

3’000 mg/kg dried foliage : **Zn**

10’000 mg/kg dried foliage : **Mn**

Adapted from Van der Ent et al (2013) Plant Soil 362: 319-334

Challenge: Improving metal phytoextraction

- Hyperaccumulators: high tissue metal concentration, but low biomass
- High yielding plants: high biomass, but moderate metal concentration

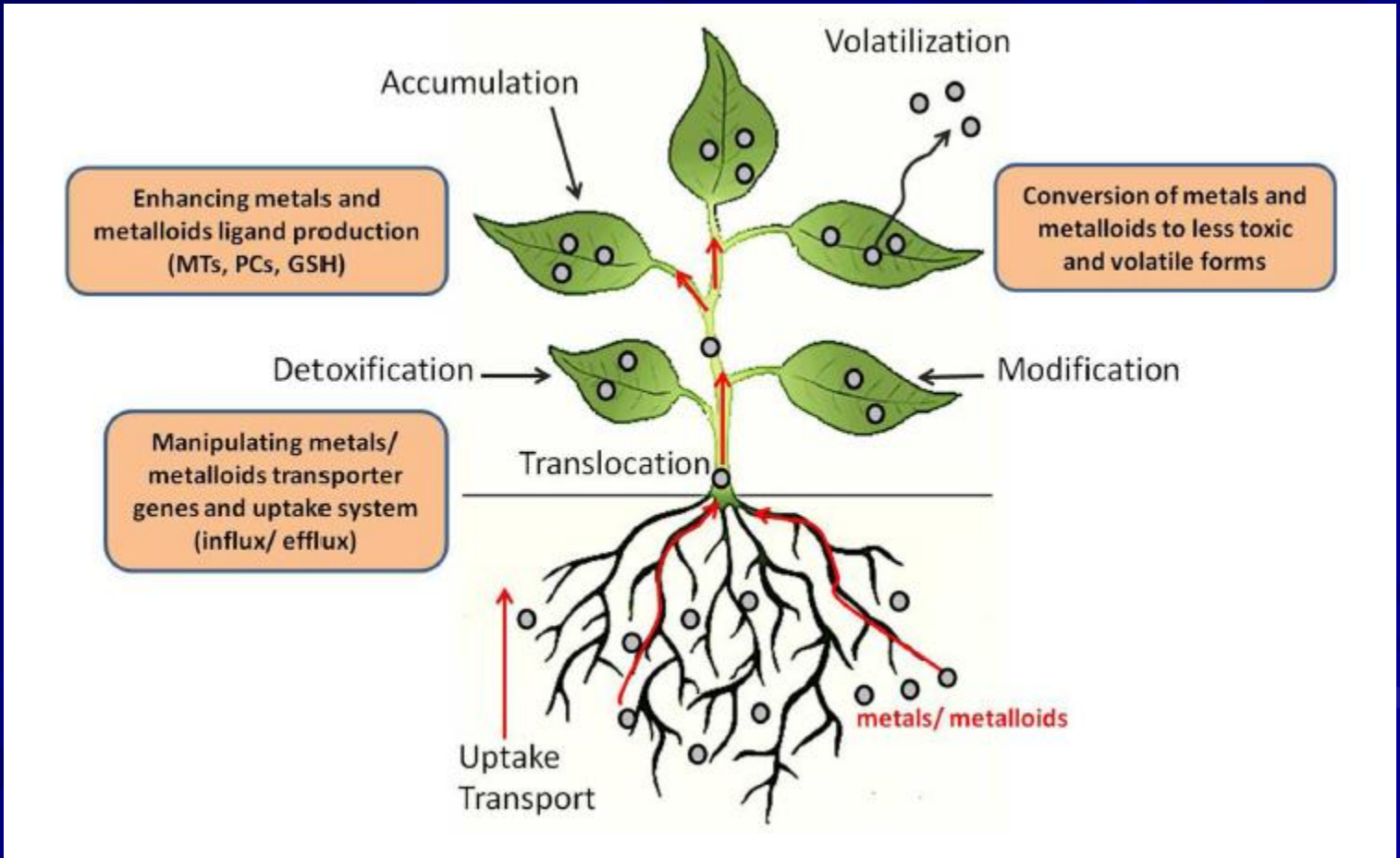
1st strategy
PLANT LEVEL

2nd strategy
SOIL LEVEL

Improvement of **plant metal accumulation** by genetic engineering or by non-GMO approaches: breeding, cultivars selection, plant density, co-cropping or inter-cropping, crop rotation, optimal harvesting time, addition of plant growth promoting bacteria

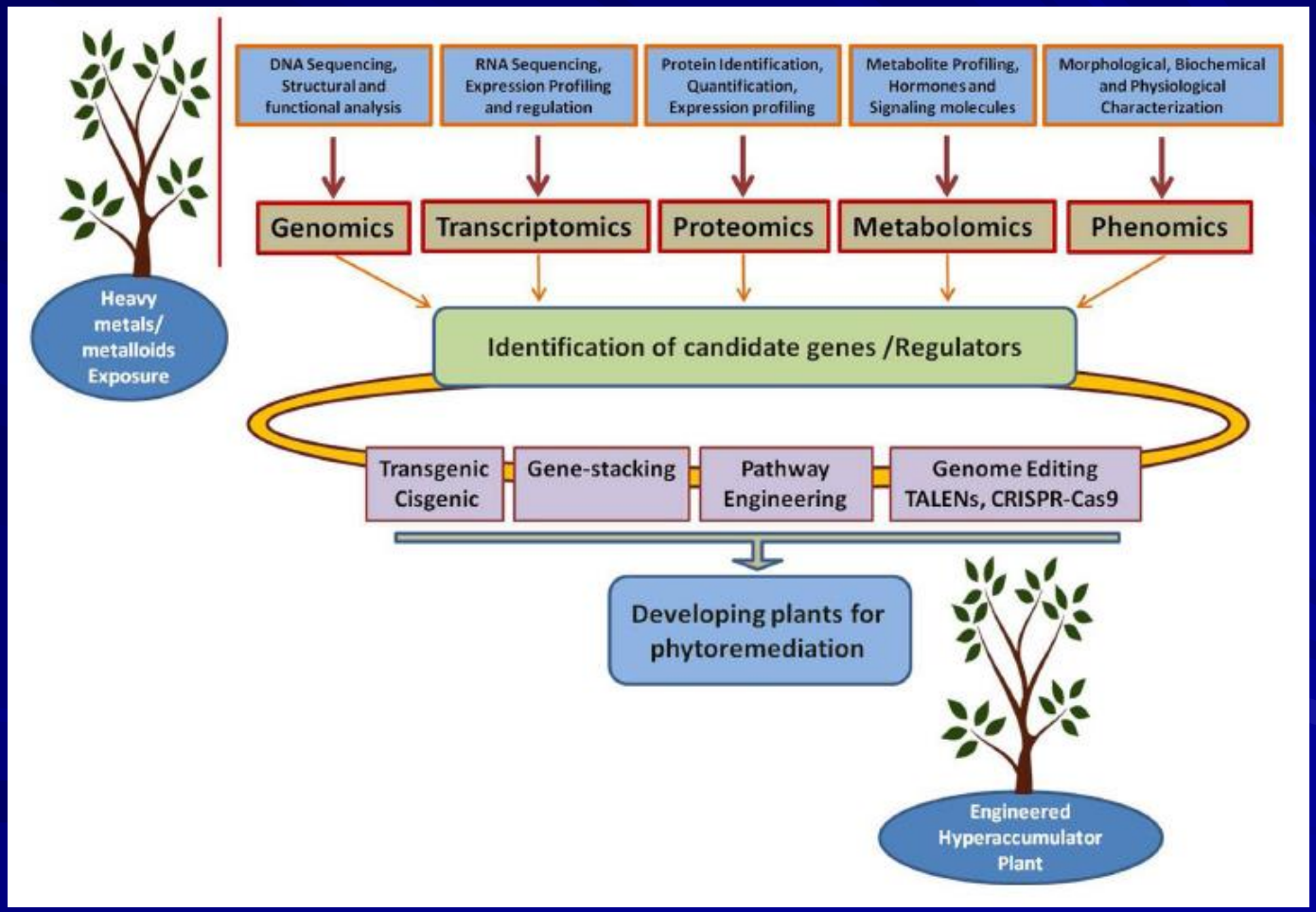
Agronomic approaches to increase **metal bioavailability** (fertilization, acidification, addition of chelating agents, amendments, bioaugmentation)

Potential biotechnological strategies for enhancing phytoextraction of metals



Mosa et al (2016) Frontiers in Plant Science 7: Art 303

Integration of «omics» tools for developing plants for phytoremediation



Mosa et al (2016) Frontiers in Plant Science 7: Art 303

A Swiss non-GMO approach to improve accumulation and extraction of heavy metals by high yielding crops

Experimental site at Rafz

Haplic Luvisol; pH 6.8; 54% sand; 28% silt; 18% clay

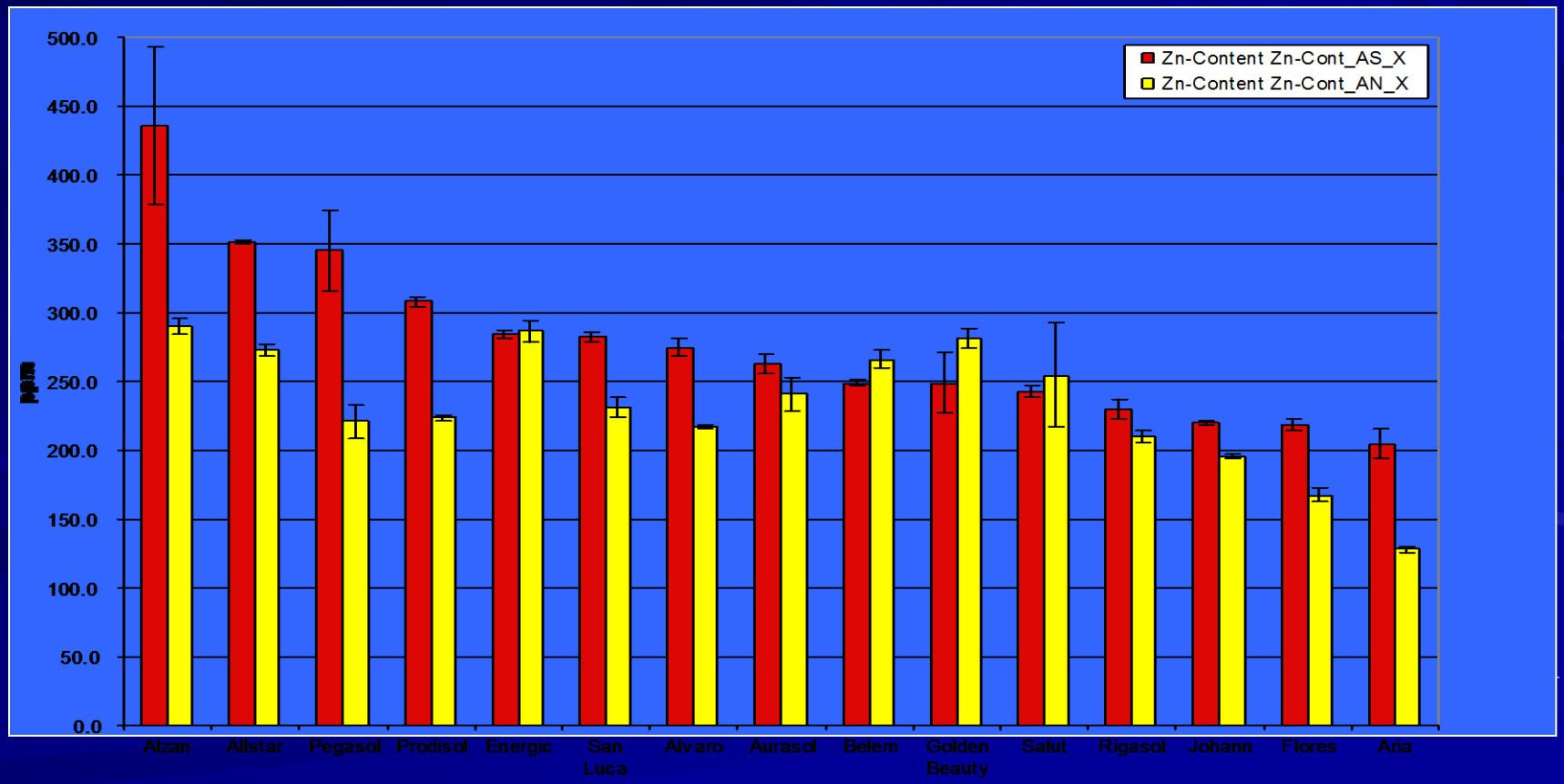
Metal concentrations (mg kg^{-1}):0.9 Cd; 58 Cu; 492 Pb; 813 Zn

Screening of efficient metal accumulating sunflowers prior to *in vitro*-breeding / mutagenesis

Nehnevajova et al. (2005, 2007, 2009) International Journal of Phytoremediation

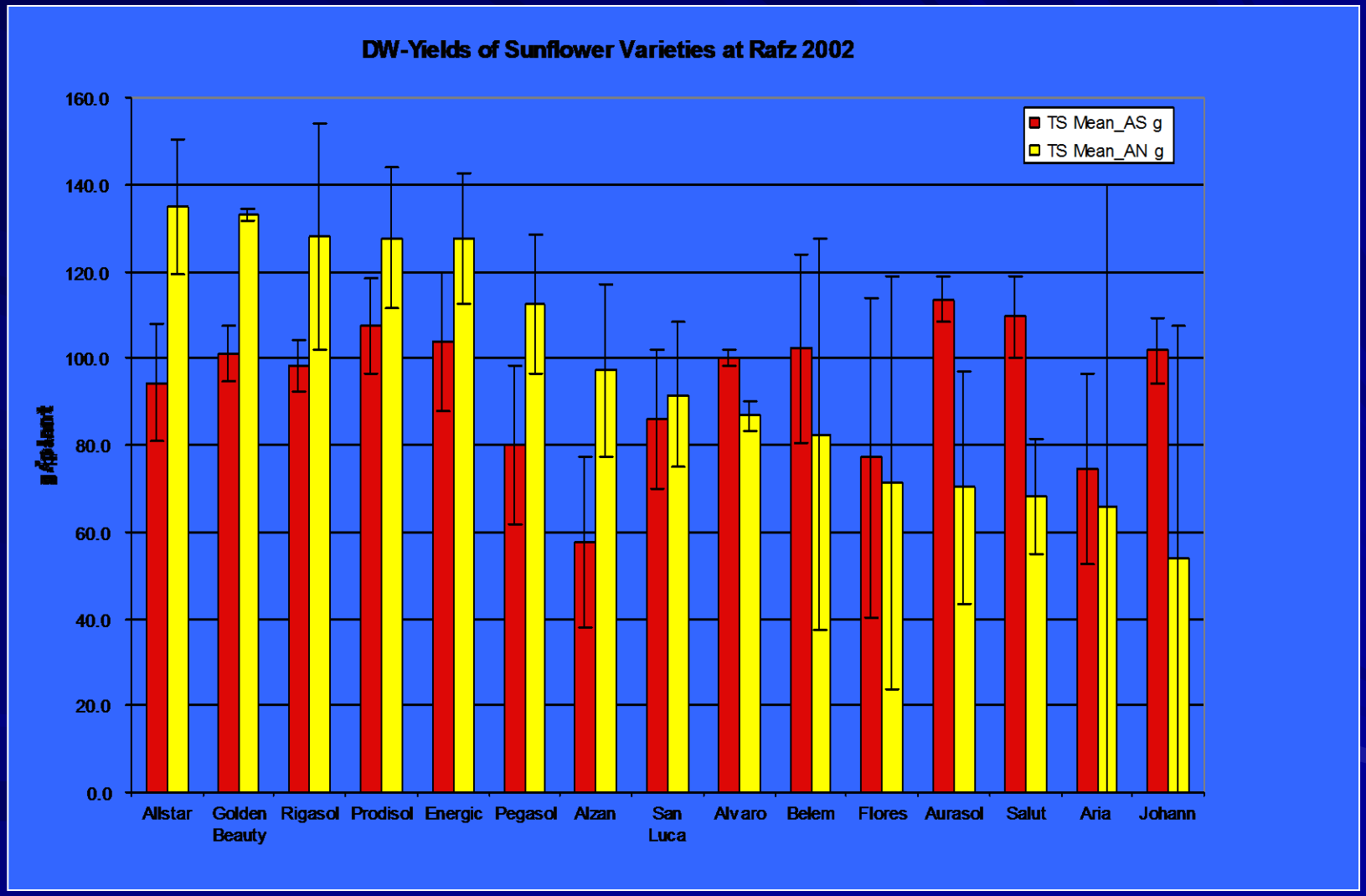
Field-based sunflower screening assessing two fertilizers / mobilizers on Zn-accumulation

- **Ammonium sulphate** fertilization instead of **ammonium nitrate**
- Selecting the best sunflower variety improves Zn-accumulation



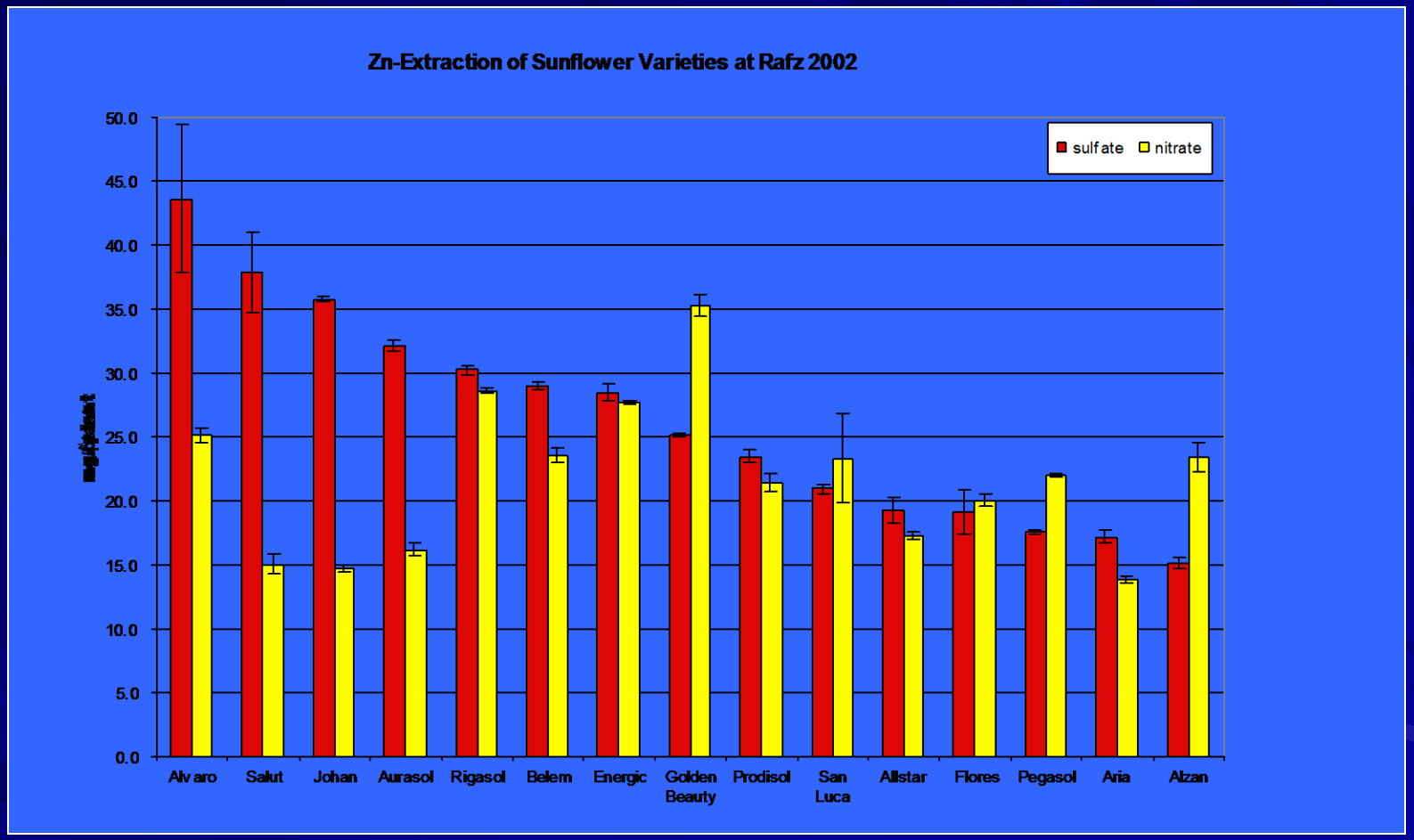
Biomass of 15 sunflower varieties

Ammonium sulphate versus **ammonium nitrate** fertilisation



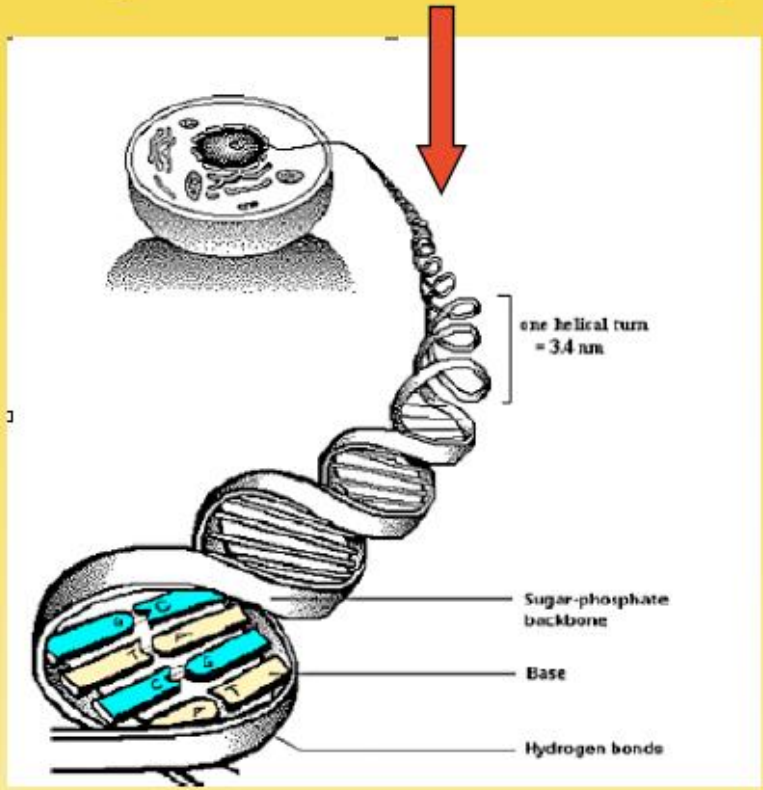
Metal extraction = Biomass x Metal accumulation

Ammonium sulphate versus **ammonium nitrate** fertilisation



Chemical mutagenesis and field-based screening of sunflower mutant generations on a metal-contaminated site

Mutagenesis with **EMS mutagen**



Goal: Sunflower mutants with enhanced biomass and metal shoot concentration and extraction

Nehnevajova et al. 2007, 2009

M_4 sunflower mutants

Rafz 2006: Screening of 10 best mutant lines of M_3 generation of 2005; totally 300 mutants

Improved M_4 sunflowers of 2006:
Best mutants produced a 4-5 times higher biomass and removed:
3.5 x more Cd, 4.7 x more Zn, 7 x more Cr, and 8 x more Pb,
as compared to non-mutagenised IBL 04 controls

2) Organic pollutants

ITRC — Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised *February 2009*

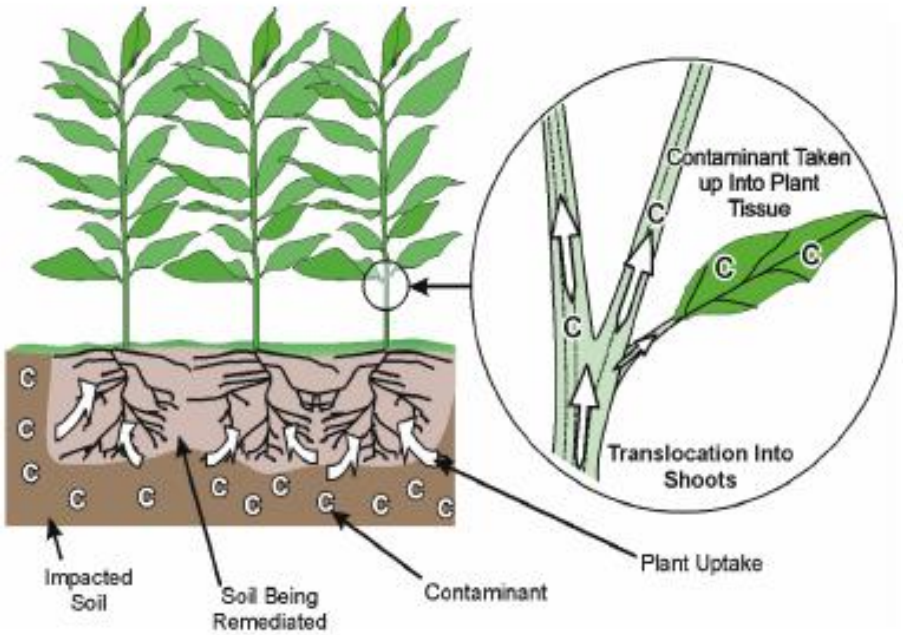
Table 1-4. Chemical properties for typical contaminants

Chemical	Log K_{ow}[a] (unitless)	Solubility[a] (mol/L)	Henry's constant[a] (unitless)	Vapor pressure[a] (atm)	TSCF[b] (unitless)	RCF[c] (L/kg)
Benzene	2.13	1.64	0.2250	0.90	0.71	3.6
Toluene	2.69	2.25	0.2760	1.42	0.74	4.5
Ethylbenzene	3.15	2.80	0.3240	1.90	0.63	6.0
m-Xylene	3.20	2.77	0.2520	1.98	0.61	6.2
Trichloroethene	2.33	2.04	0.4370	1.01	0.74	3.9
Aniline	0.90	0.41	2.2×10^5	2.89	0.26	3.1
Nitrobenzene	1.83	1.77	0.0025	3.68	0.62	3.4
Phenol	1.45	0.20	$>1.0 \times 10^5$	3.59	0.47	3.2
Pentachlorophenol	5.04	4.27	1.5×10^4 [(d)]	6.75[d]	0.07	54
Atrazine	2.69	3.81	1.0×10^7 [(d)]	9.40[d]	0.74	4.5
1,2,4-Trichlorobenzene	4.25	3.65	0.1130	3.21	0.21	19
RDX[e]	0.87	4.57	-	-	0.25	3.1

2.1) Phytoextraction of PCBs: A Case Study

Ken Reimer *, Melissa Whitfield,
Barbara Zeeb, and Allison Rutter

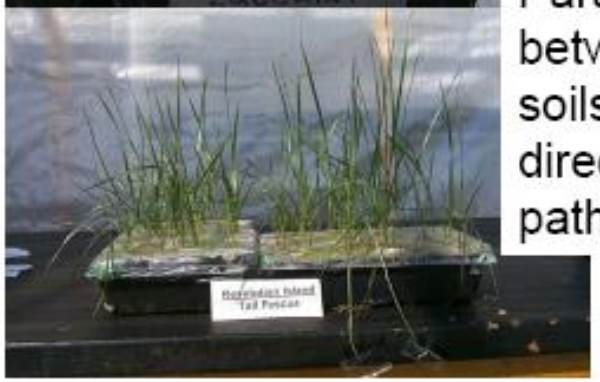
Phytoextraction



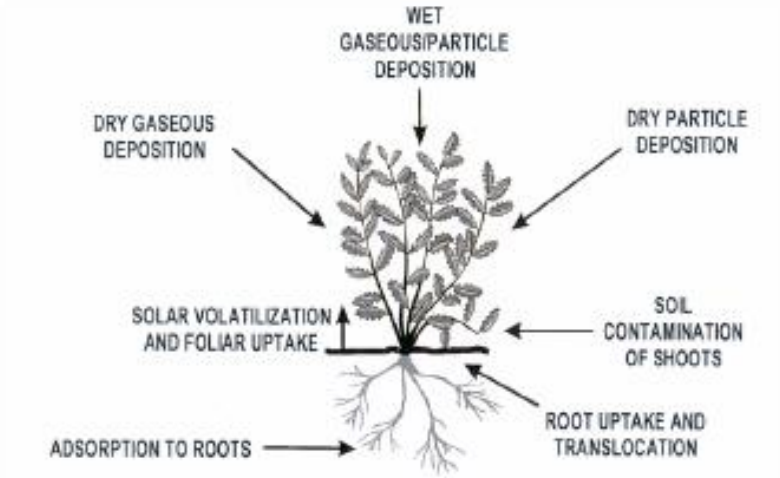
- Previously thought that hydrophobic organochlorines (log K_{OW} >5) could not be extracted
- Now known that this is possible for compounds like DDT, PCBs

Controlled GH Studies

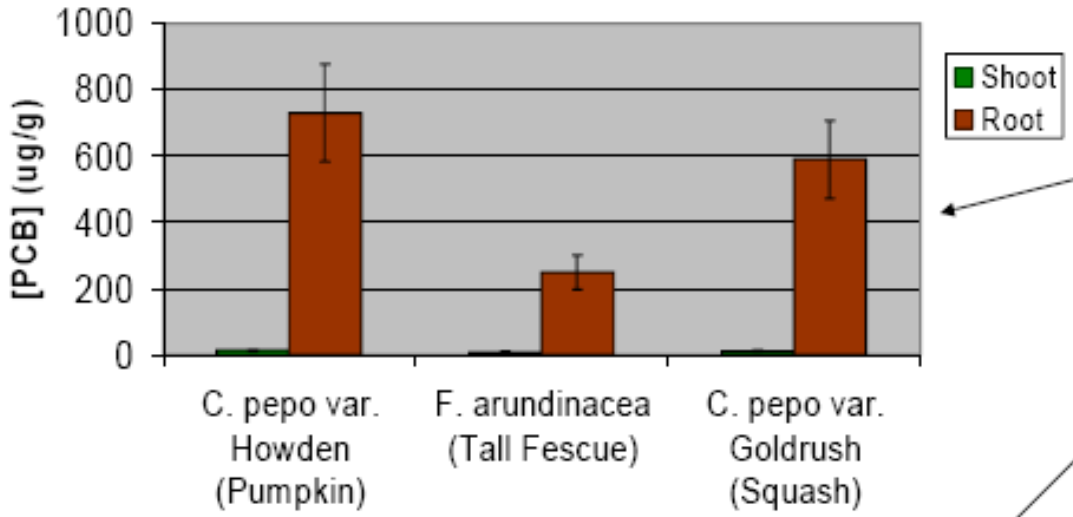
To examine potential for PCB phytoextraction



Parafilm seal between shoots & soils to eliminate direct contact pathways



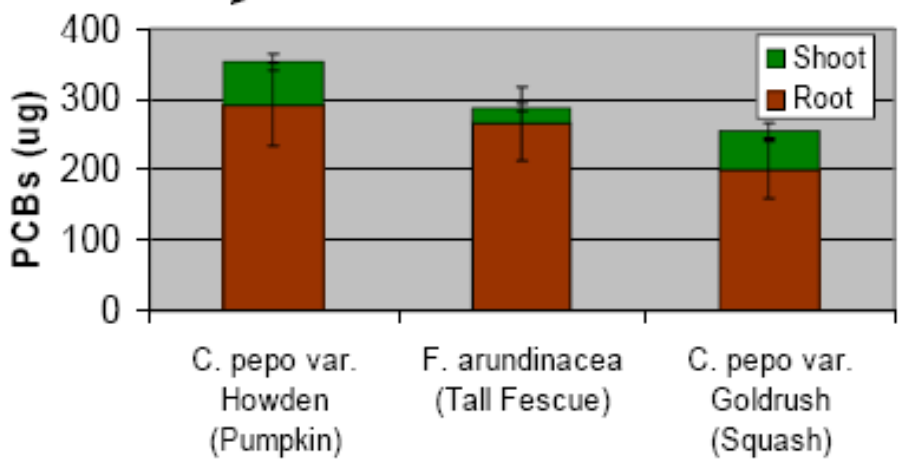
Greenhouse Results



PCB-Concentration (ug/g)

vs.

PCB-Extraction (ug)



Ex-Situ Field Plots

Scaled-up,outdoor experiments

-same soil as greenhouse work

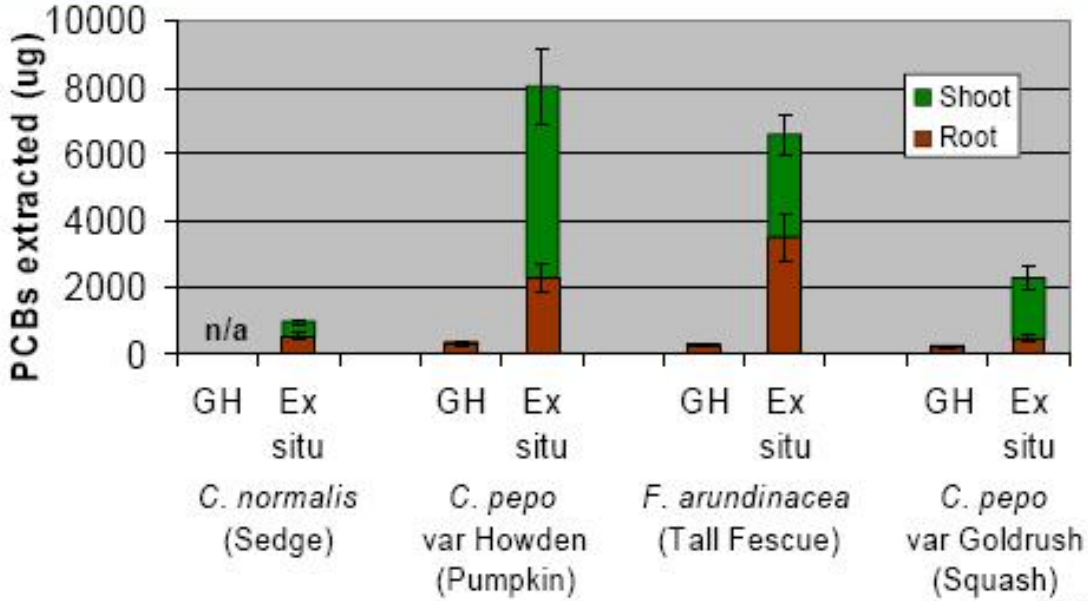
Safety considerations:

- Registered PCB storage facility
- Water collection and treatment
- Spill barrier
- Weekly air monitoring

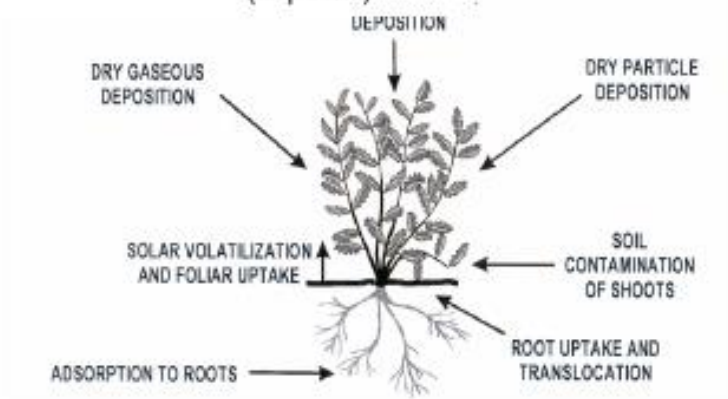
Species used:

- Pumpkin, Squash, Tall Fescue, and Sedge

Greenhouse vs. Ex situ Field Results

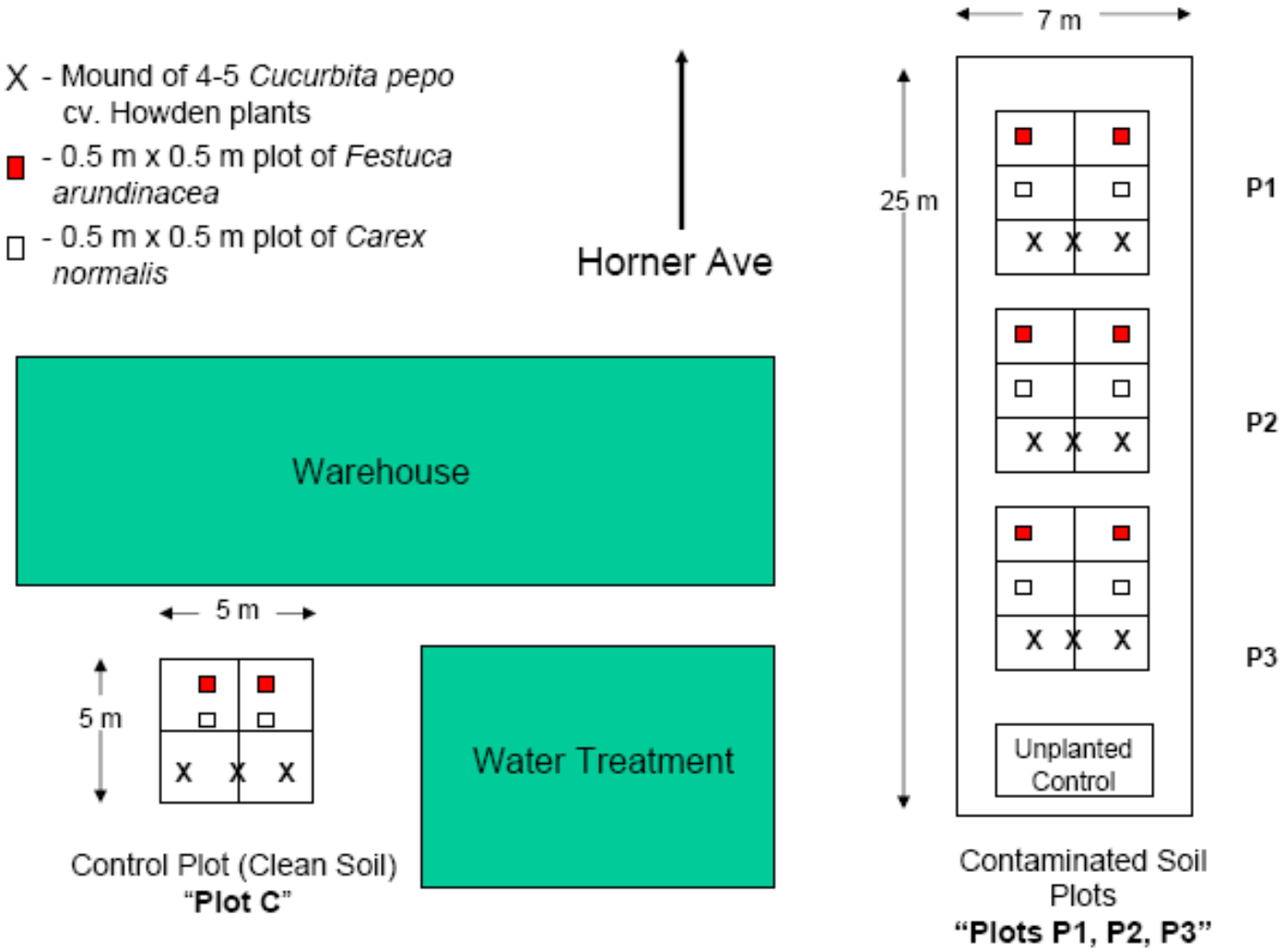


PCB-Extraction (ug)

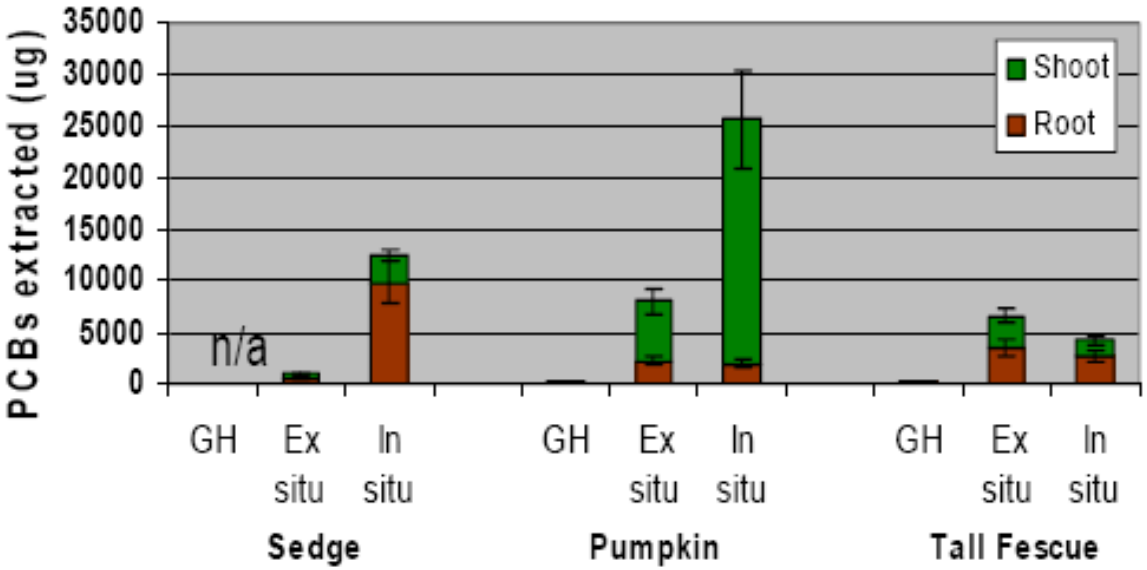


$[PCB]_{soil}$ = ~500 ug/g

Experimental Design



Greenhouse vs. Ex situ Field vs. In situ Field

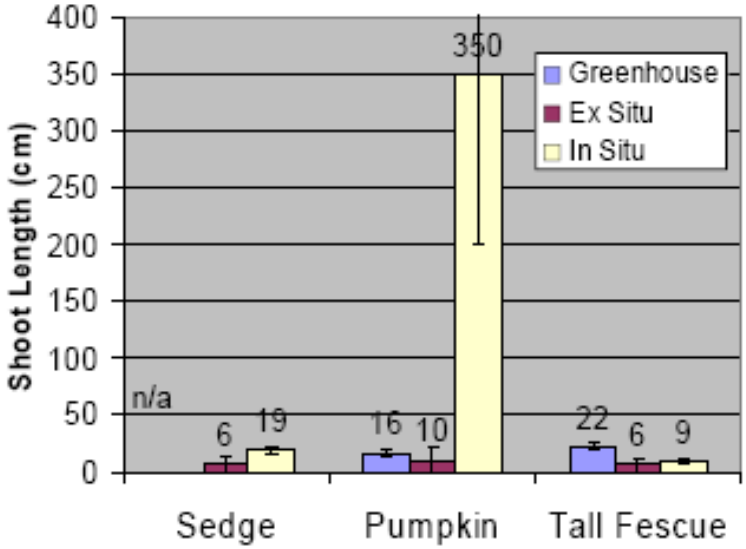


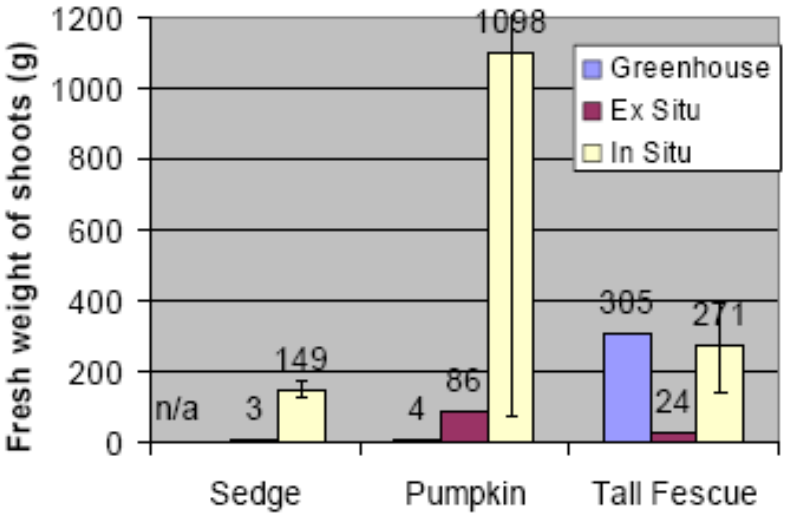
PCB-Extraction (ug)

- Increased biomass *in situ* resulted in large increases in total PCB uptake

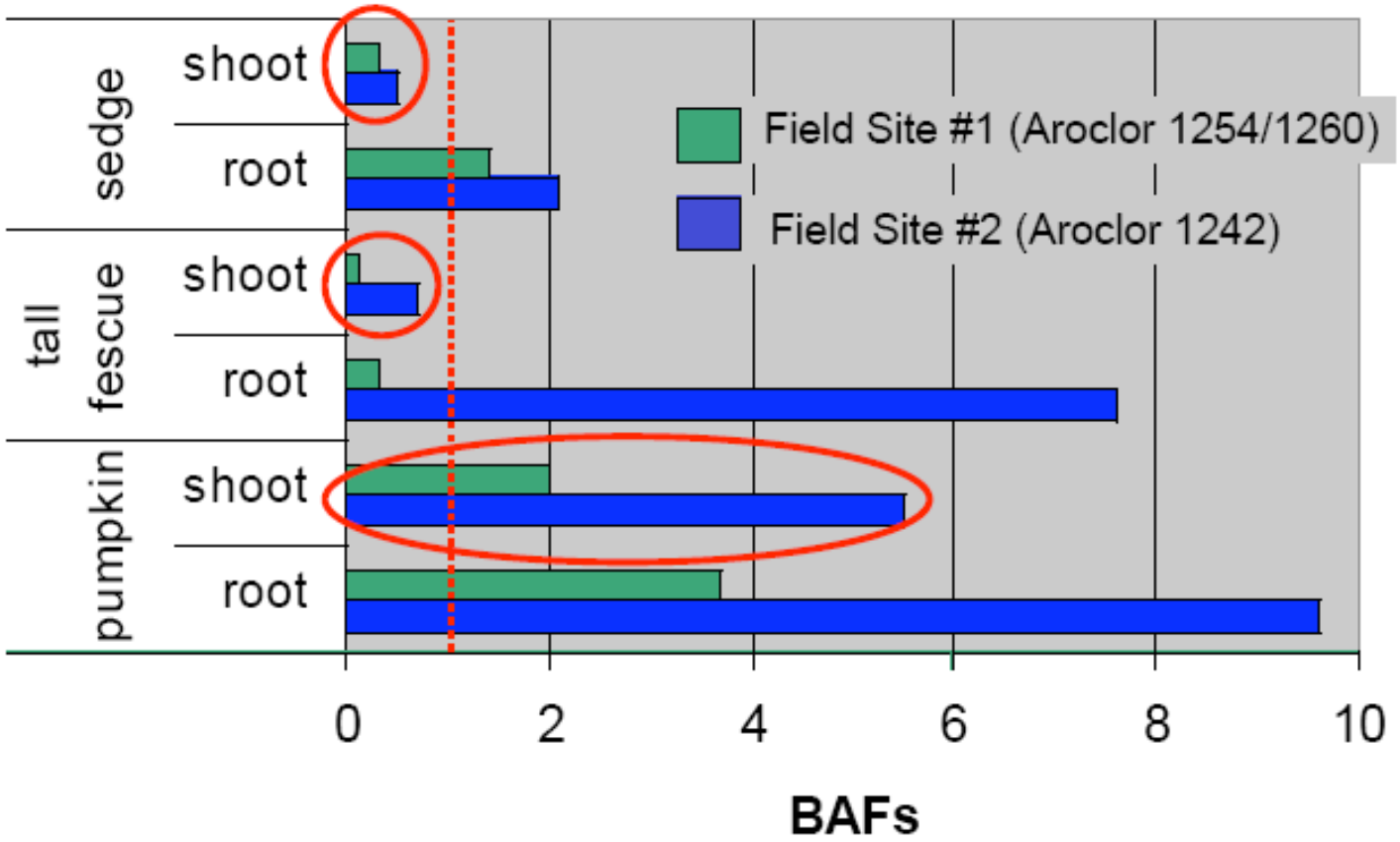
Greenhouse vs. Ex situ Field vs. In situ Field

	Soil [PCB]	Aroclor
Greenhouse	120 – 390 ug/g, median 170 ug/g	1260
Ex situ	410 – 570 ug/g, median 520 ug/g	1260
In situ	2 – 210 ug/g, median 30 ug/g	1254/1260



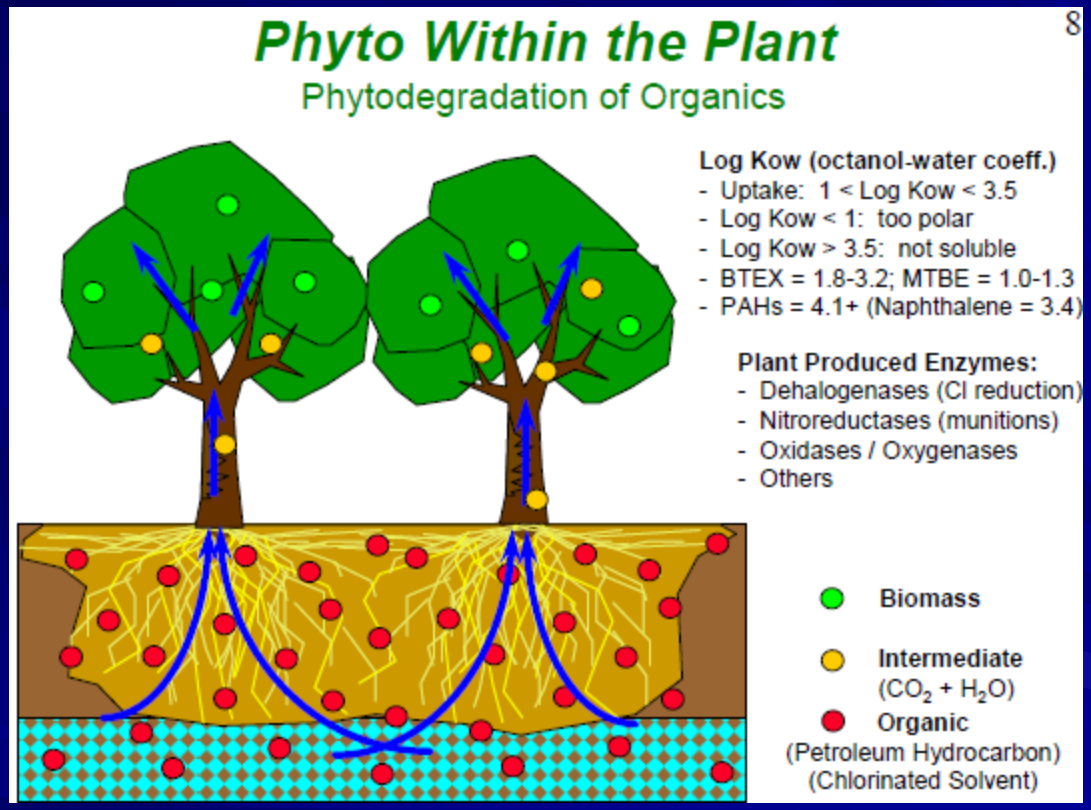


Bioaccumulation Factor (BAF) Comparison

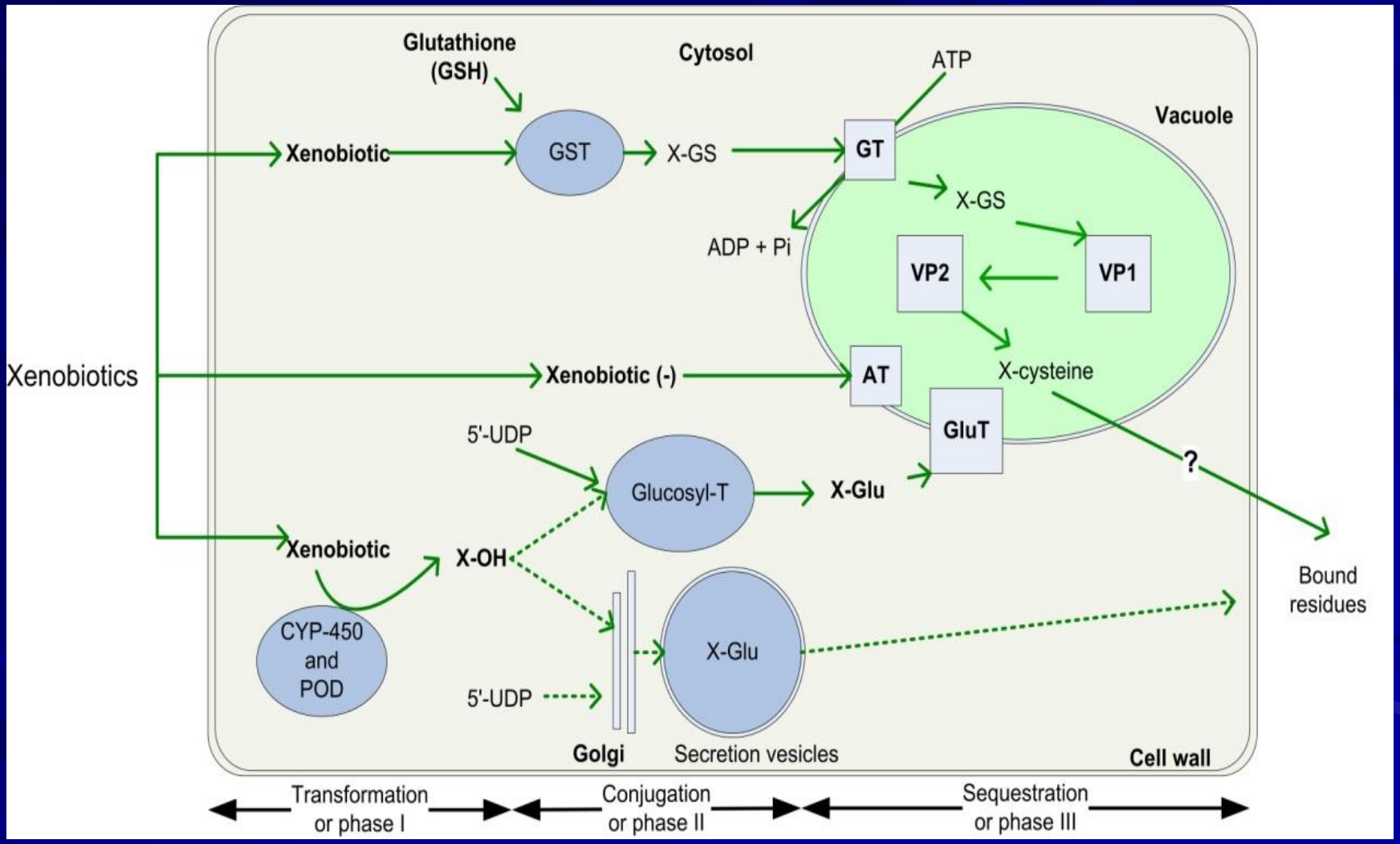


- Exudation of organic acids (citrate, malate) helps desorbing and uptake
- Major latex-like proteins and zinc finger family proteins are involved in translocation

2.2) Phytodegradation

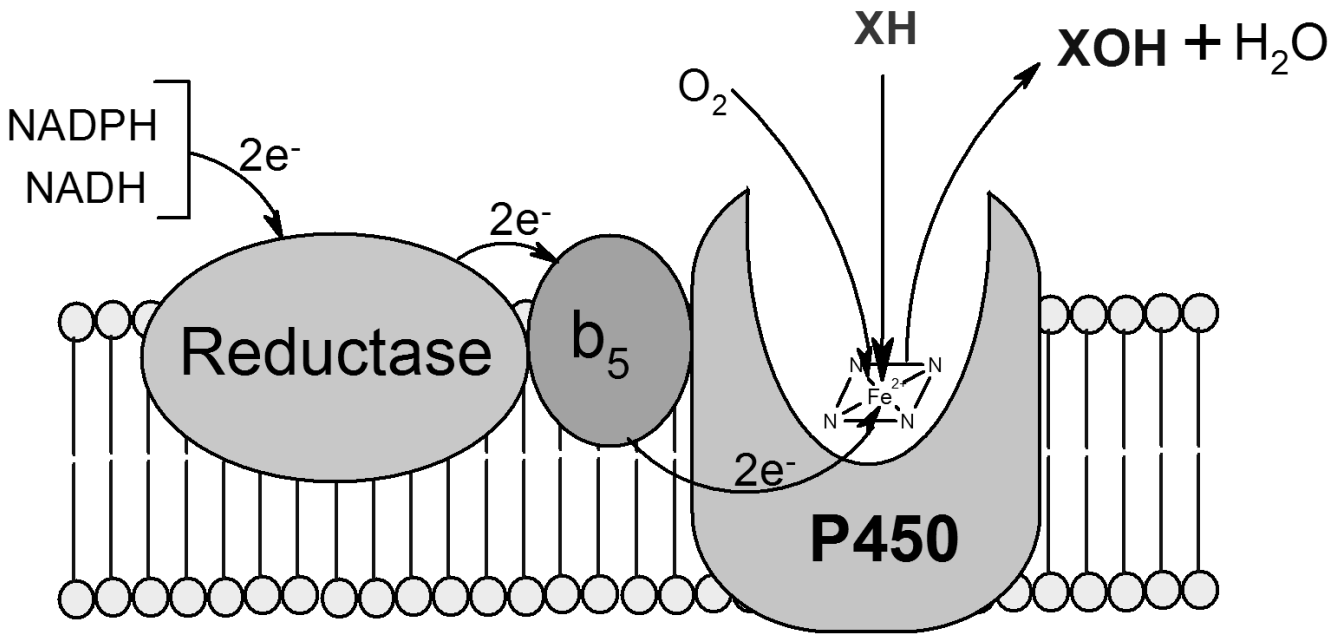


Main detoxification pathways of xenobiotics in plant cells



Coleman et al. (1997) Trends Plant Sci 2:144-151

The role of Cytochrome P450 mono-oxygenase in hydroxylation of xenobiotic compounds



XH - nonpolar xenobiotic

XOH - polar product of hydroxylation

Kvesitadze et al (2006)

Measure of oxygen consumption by fluorimetry

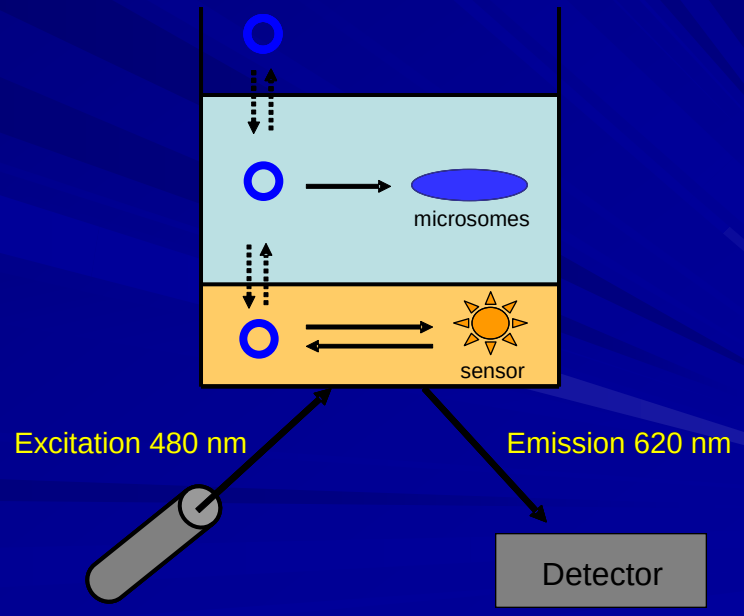
Oxygen-sensitive fluorophore incorporated in a permeable silicone rubber matrix at the bottom of each well

Oxygen diffusion into plate well from atmosphere

Diffusion into medium

Consumption by cytochromes

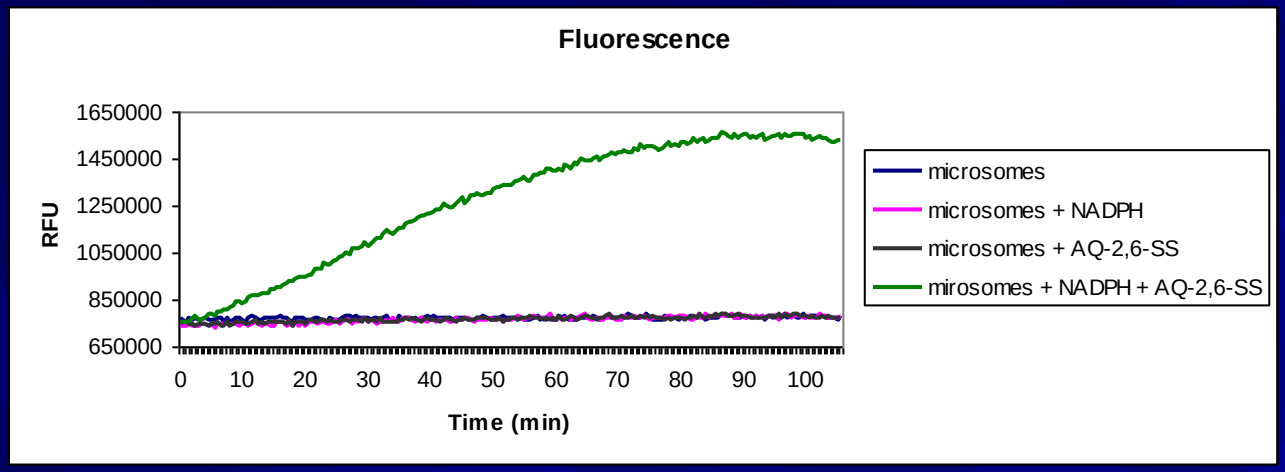
Oxygen quenches fluorescence from the sensor at the well bottom

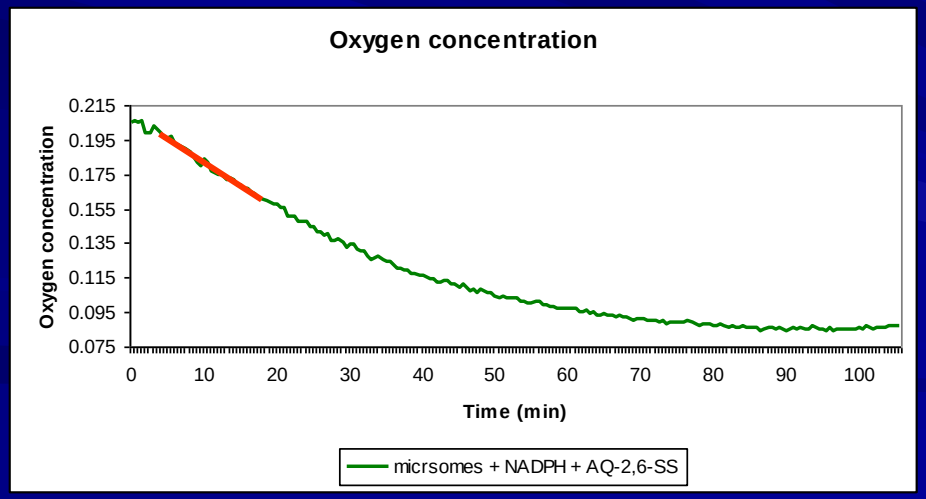


Oxygen decrease → fluorescence increase

Measure of oxygen consumption by fluorimetry

Olry et al. (2007) Plant Journal 51:331-340

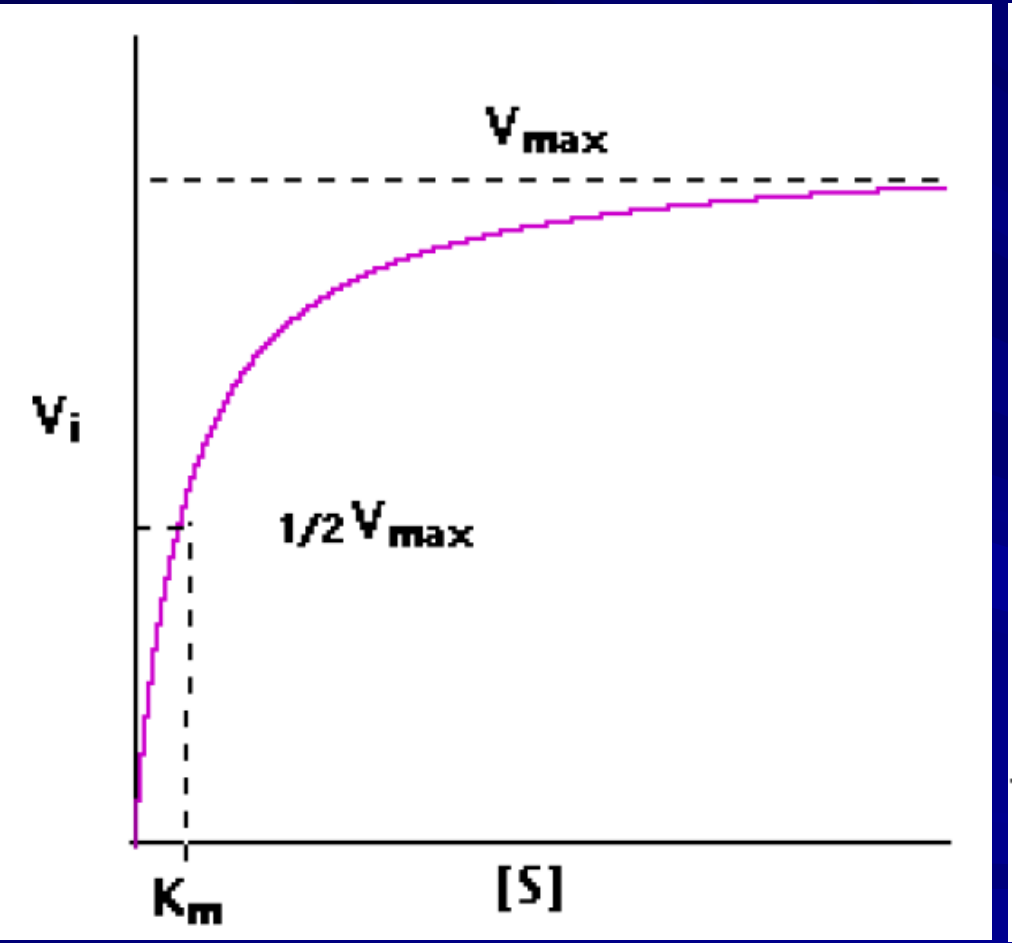


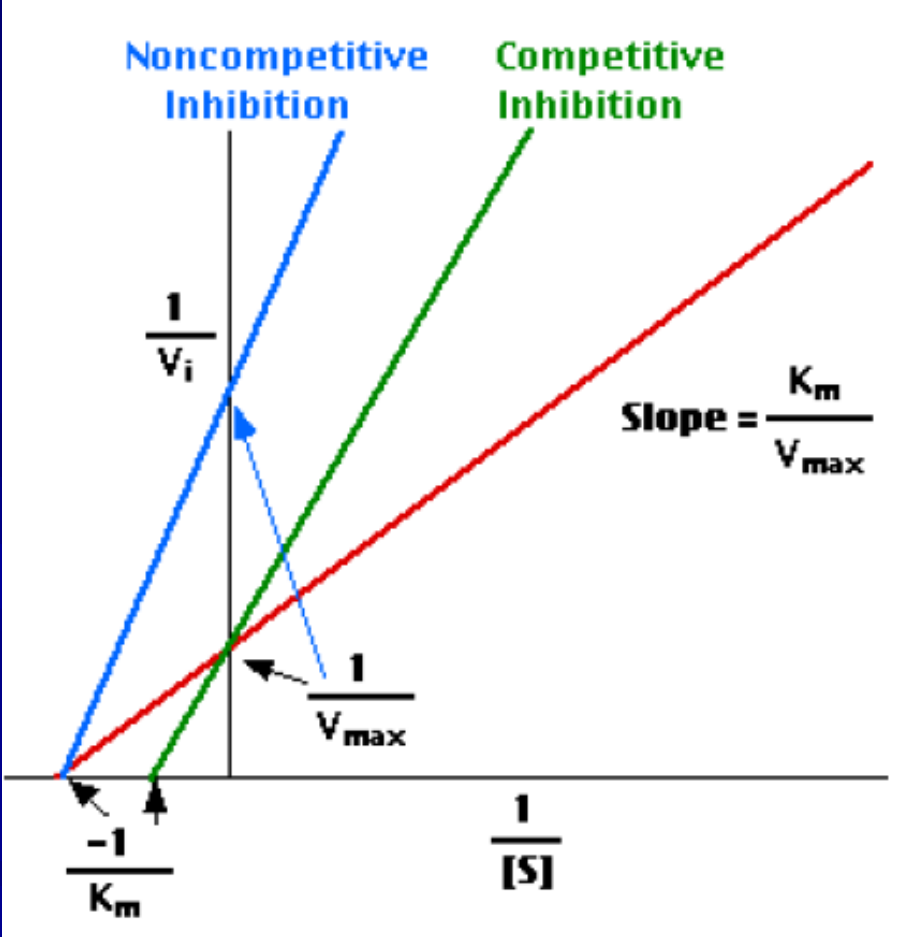


Enzymatic activity

Page and Schwitzguébel (2009) Plant Cell Rep 28:1725-1735; Environ Sci Pollut Res 16:805-816

Enzyme kinetics are of utmost importance





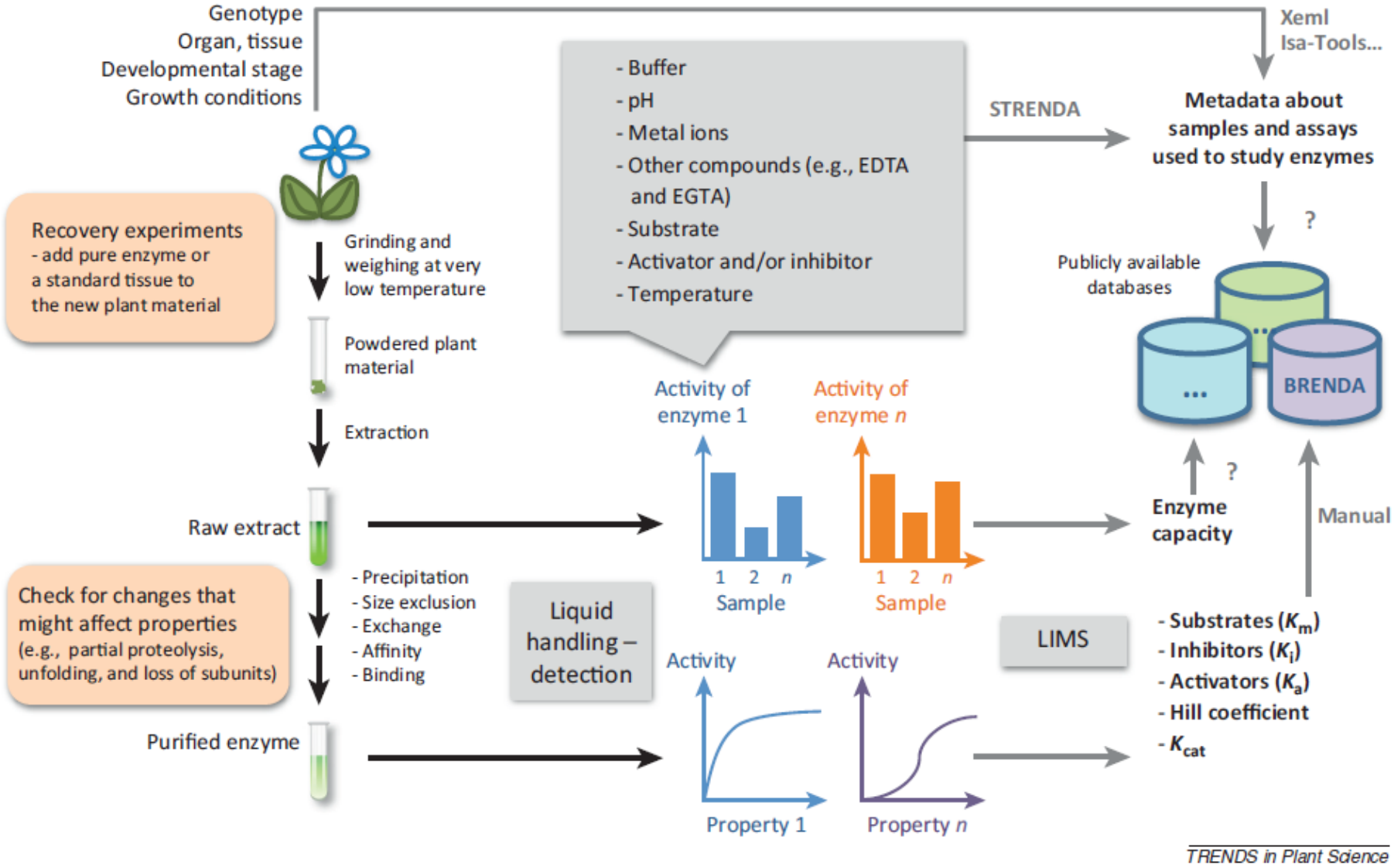


Figure I. Analytical pipeline for activity measurements.

Stitt and Gibon (2014) Trends Plant Sci 19:256-265

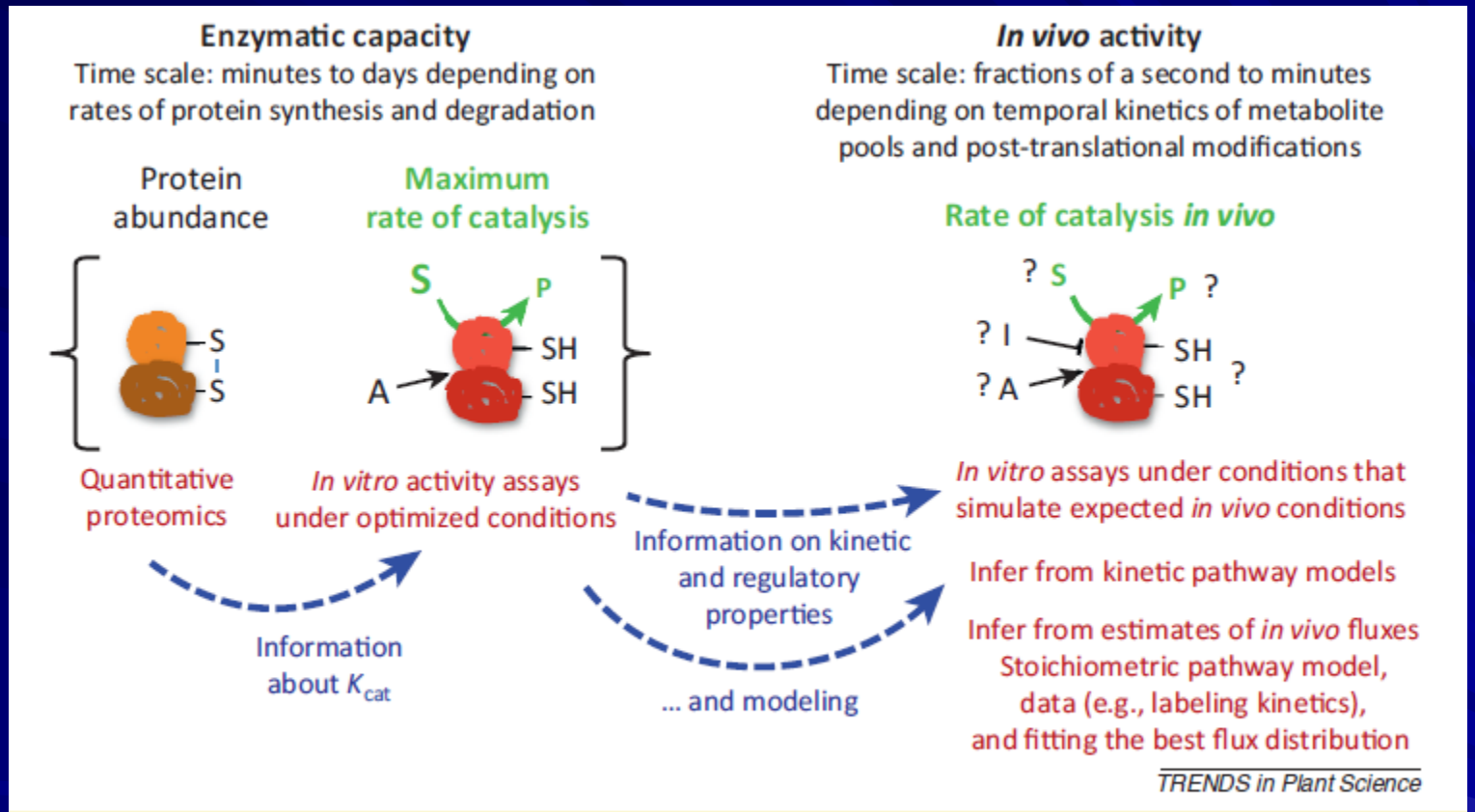
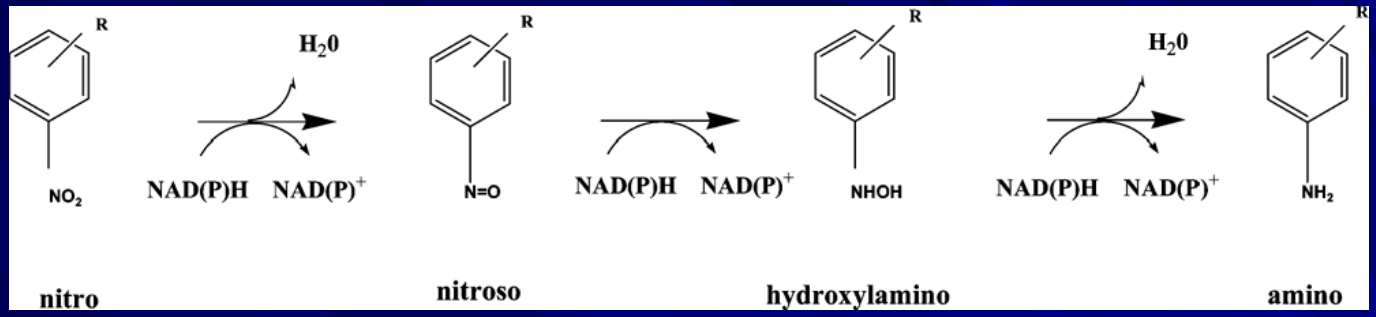
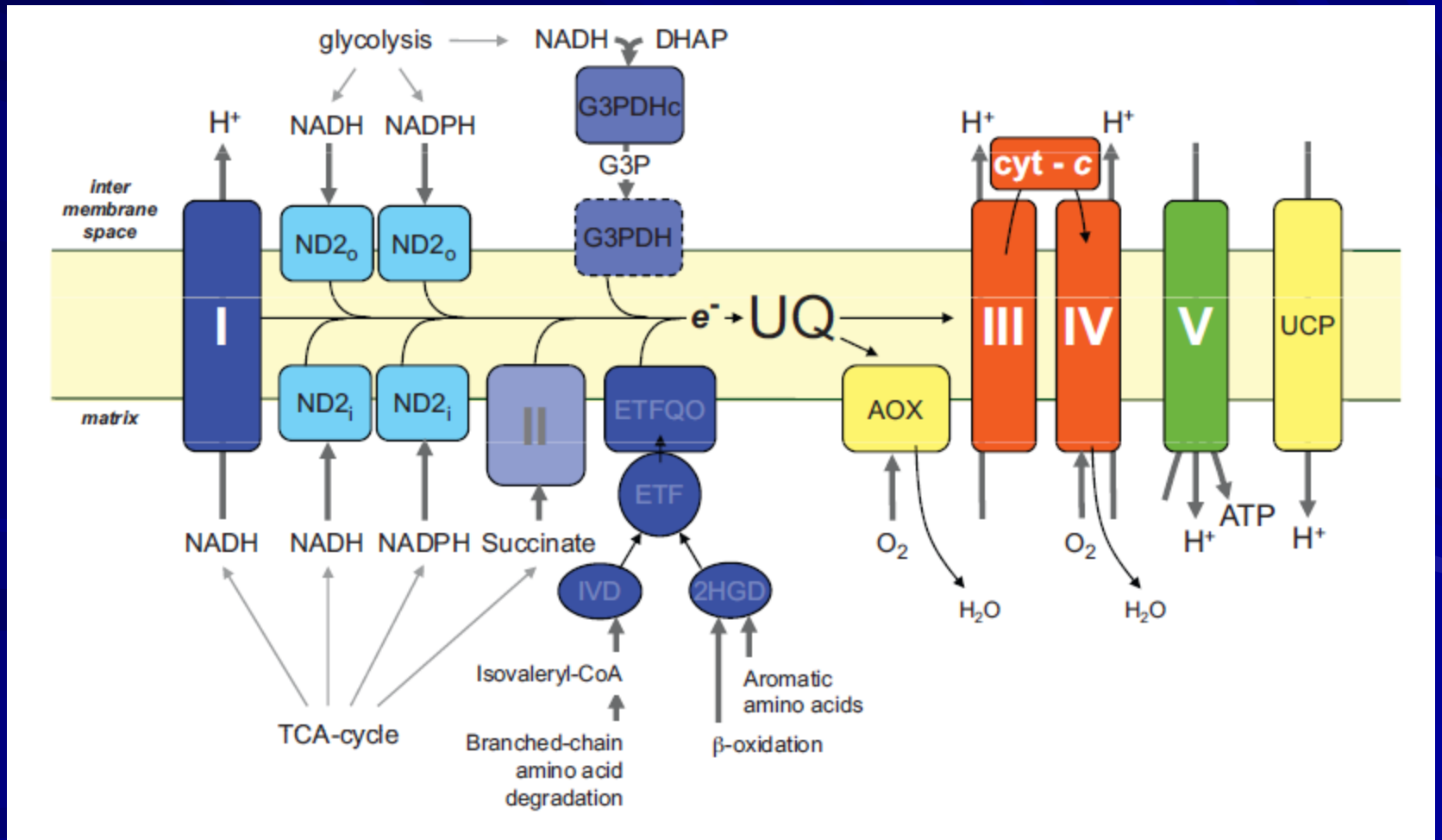


Figure II. Relation between protein abundance, catalytic capacity, and the *in vivo* rate of catalysis.

Stitt and Gibon (2014) Trends Plant Sci 19: 256-265

Nitroaromatic compounds reduction





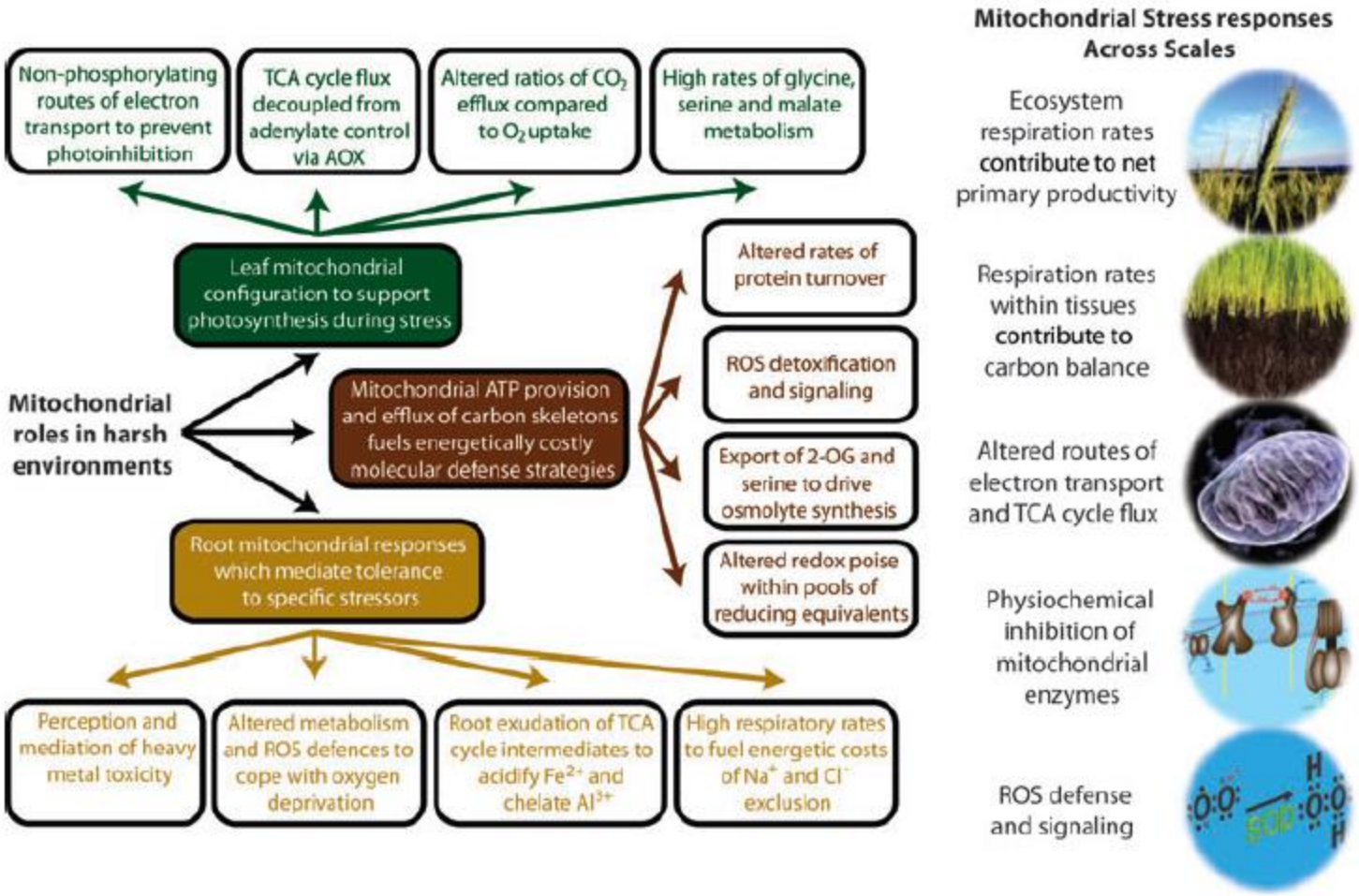
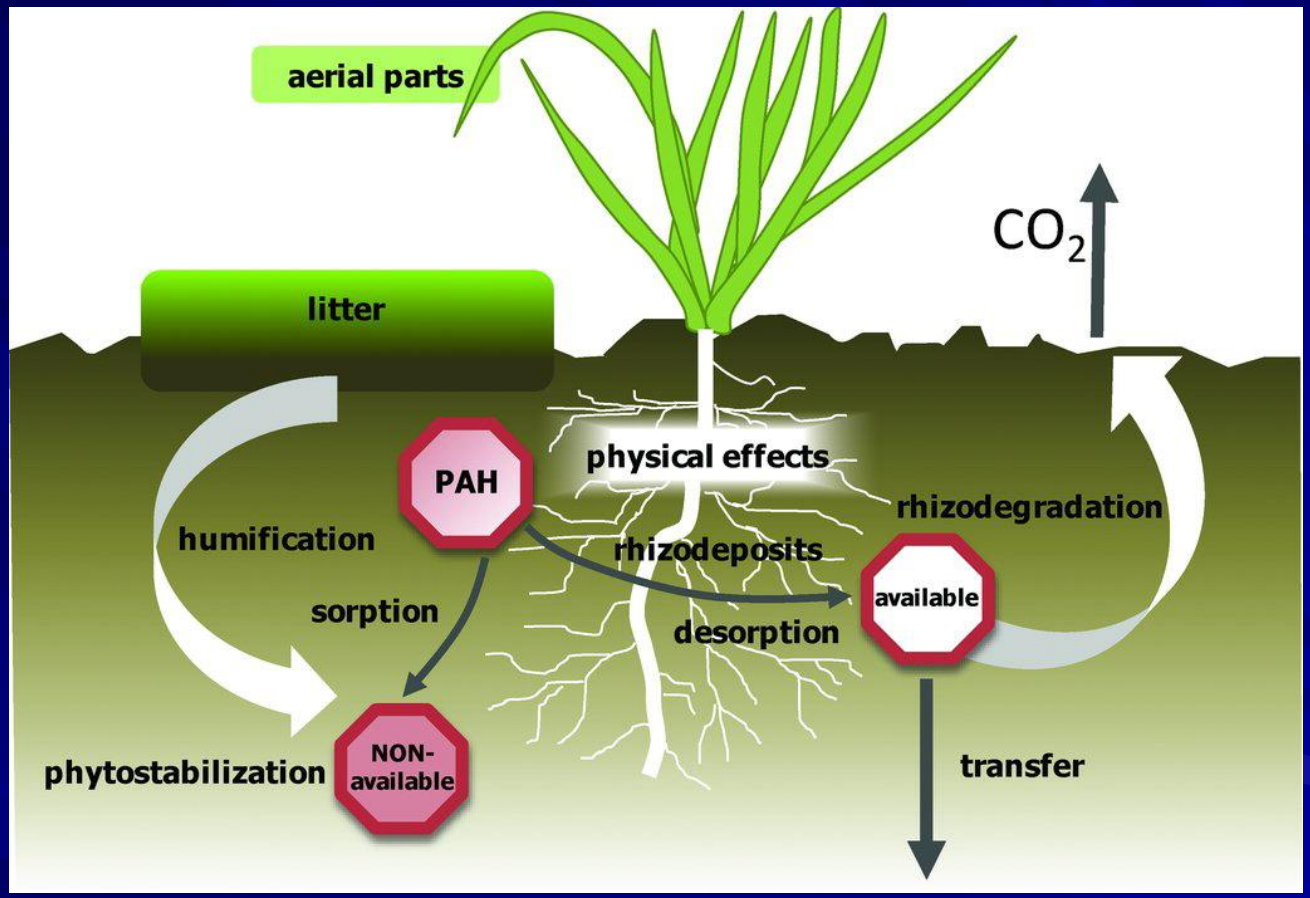


Figure 4. Plant mitochondrial roles in harsh environments.

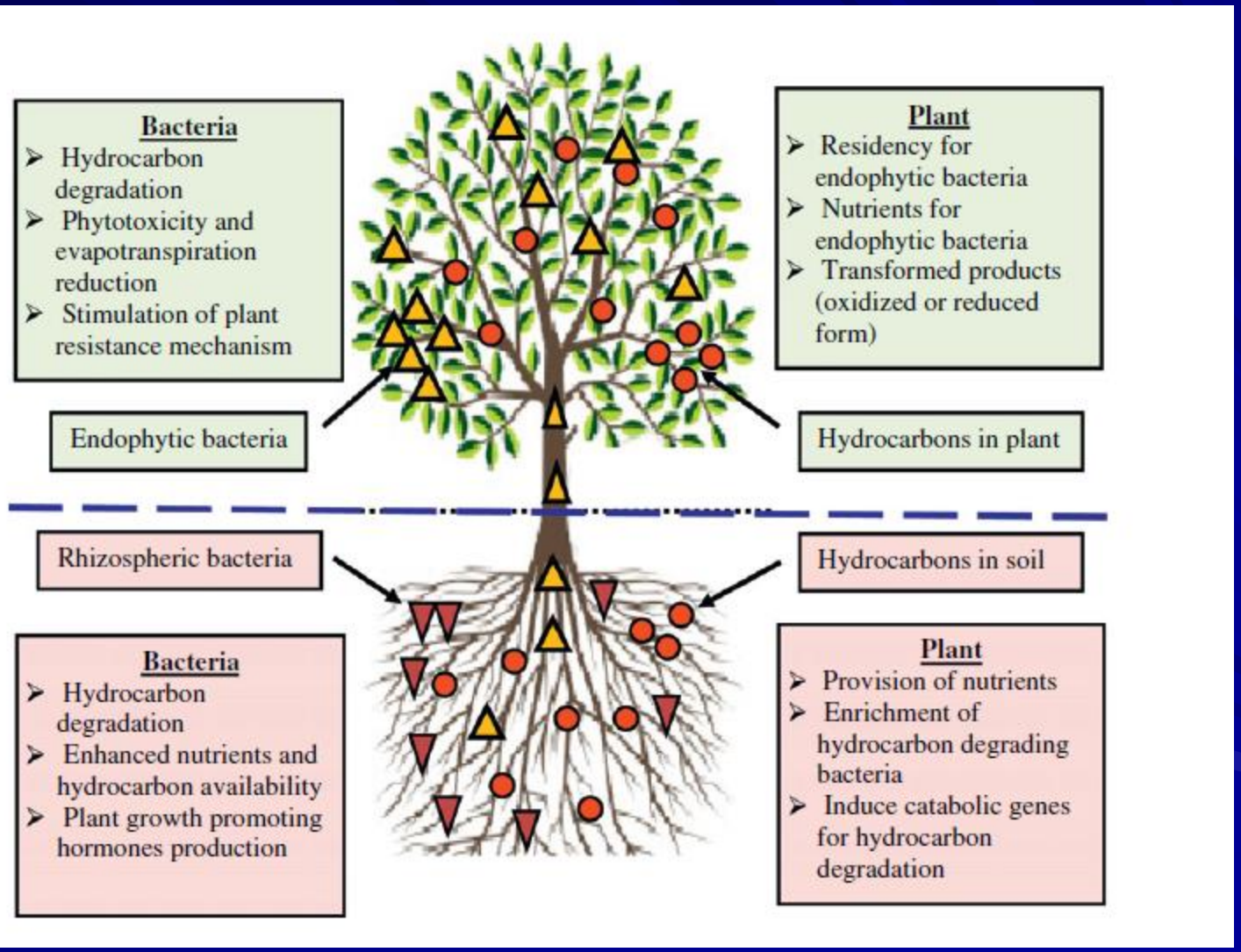
Jacoby et al (2012)

2.3) Phytostimulation (rhizodegradation)



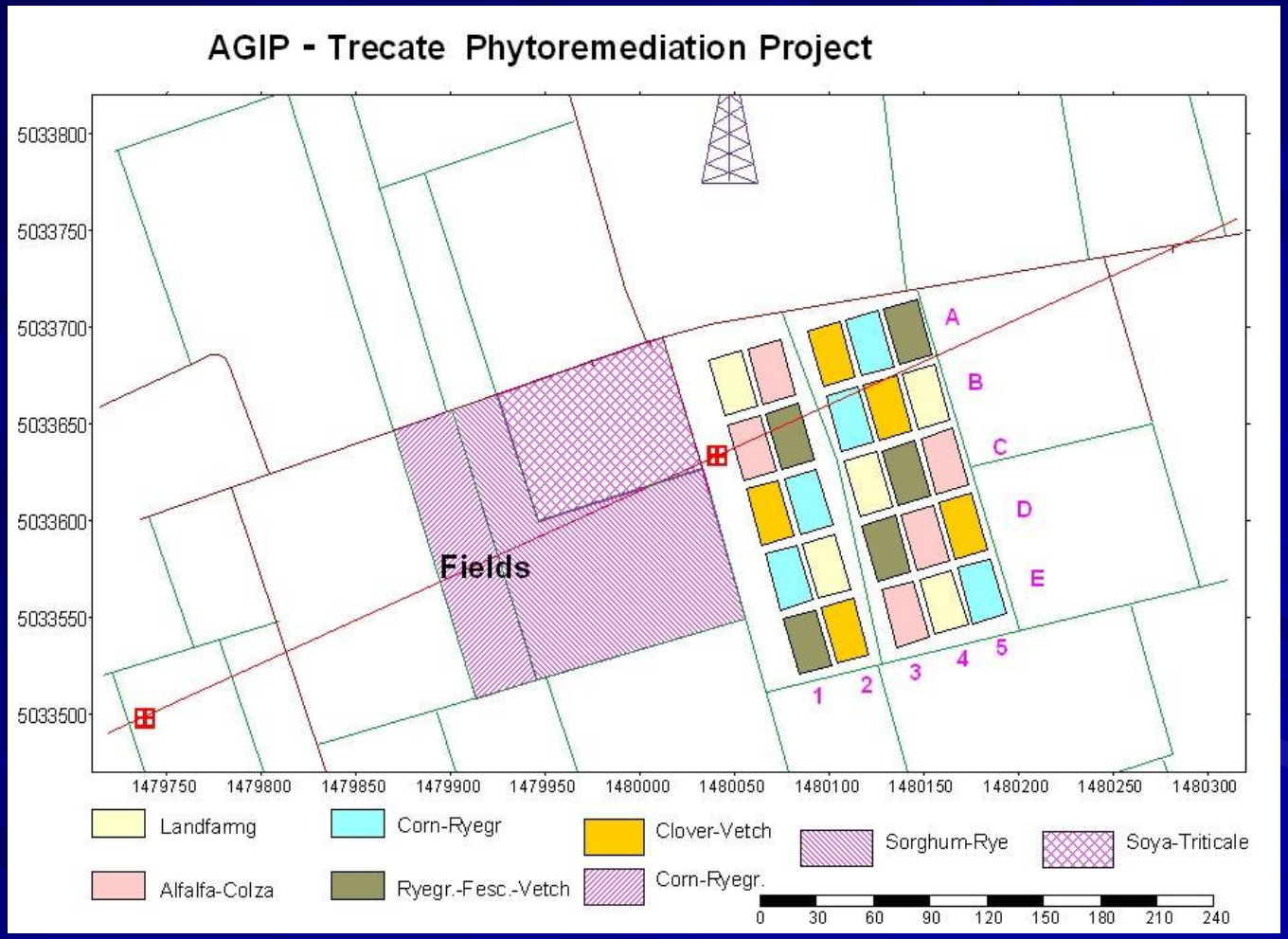
Ouvrard et al (2013) Biodegradation 24:539-548

Plant-bacteria partnerships for the remediation of hydrocarbons contaminated soil



Khan et al. (2013) Chemosphere 90:1317-1332

Petroleum hydrocarbons: a success story

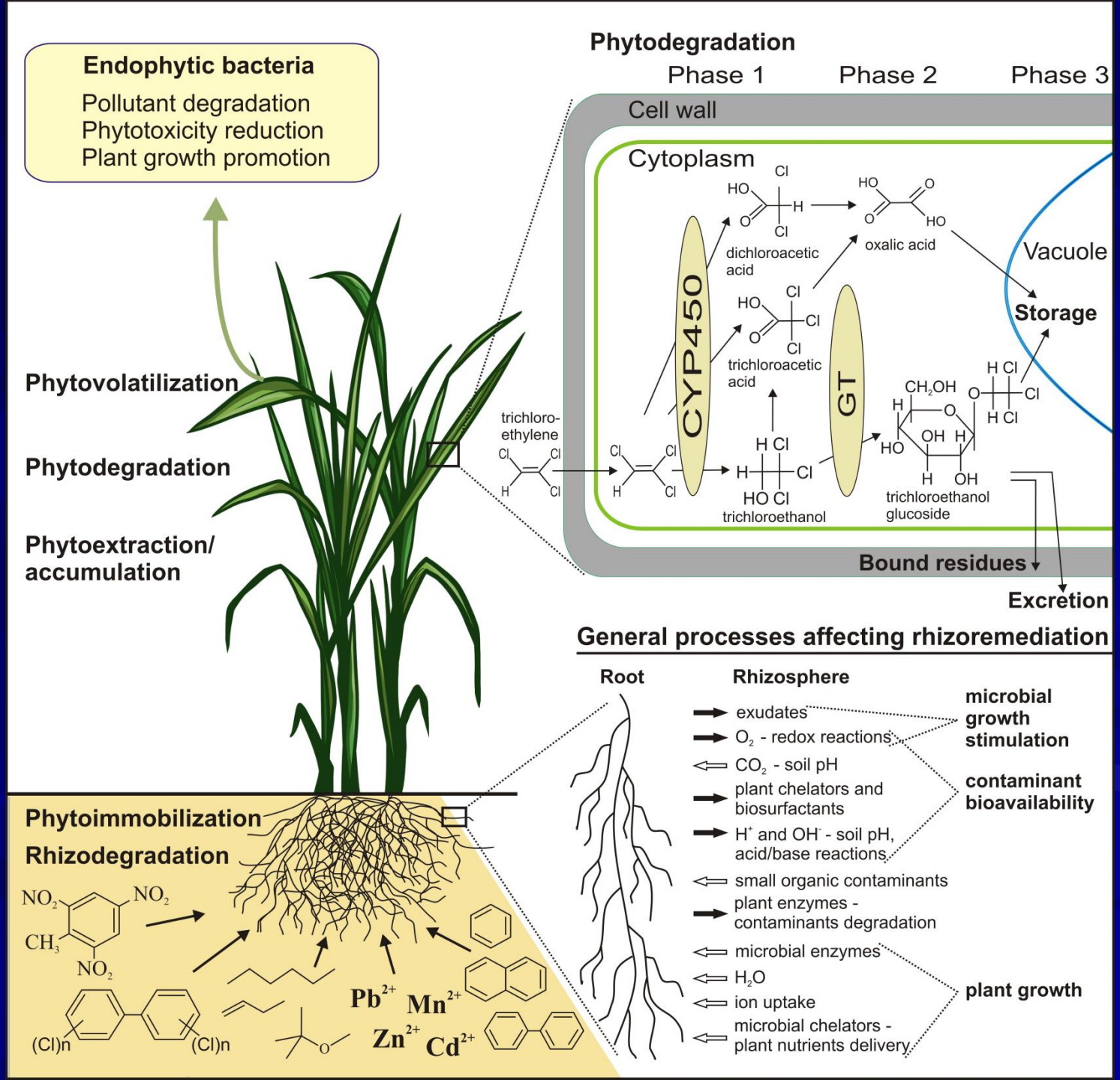


Schwitzguébel et al (2011) Environ Sci Pollut Res 18:842-856

Schwitzguébel et al (2011) Environ Sci Pollut Res 18:842-856

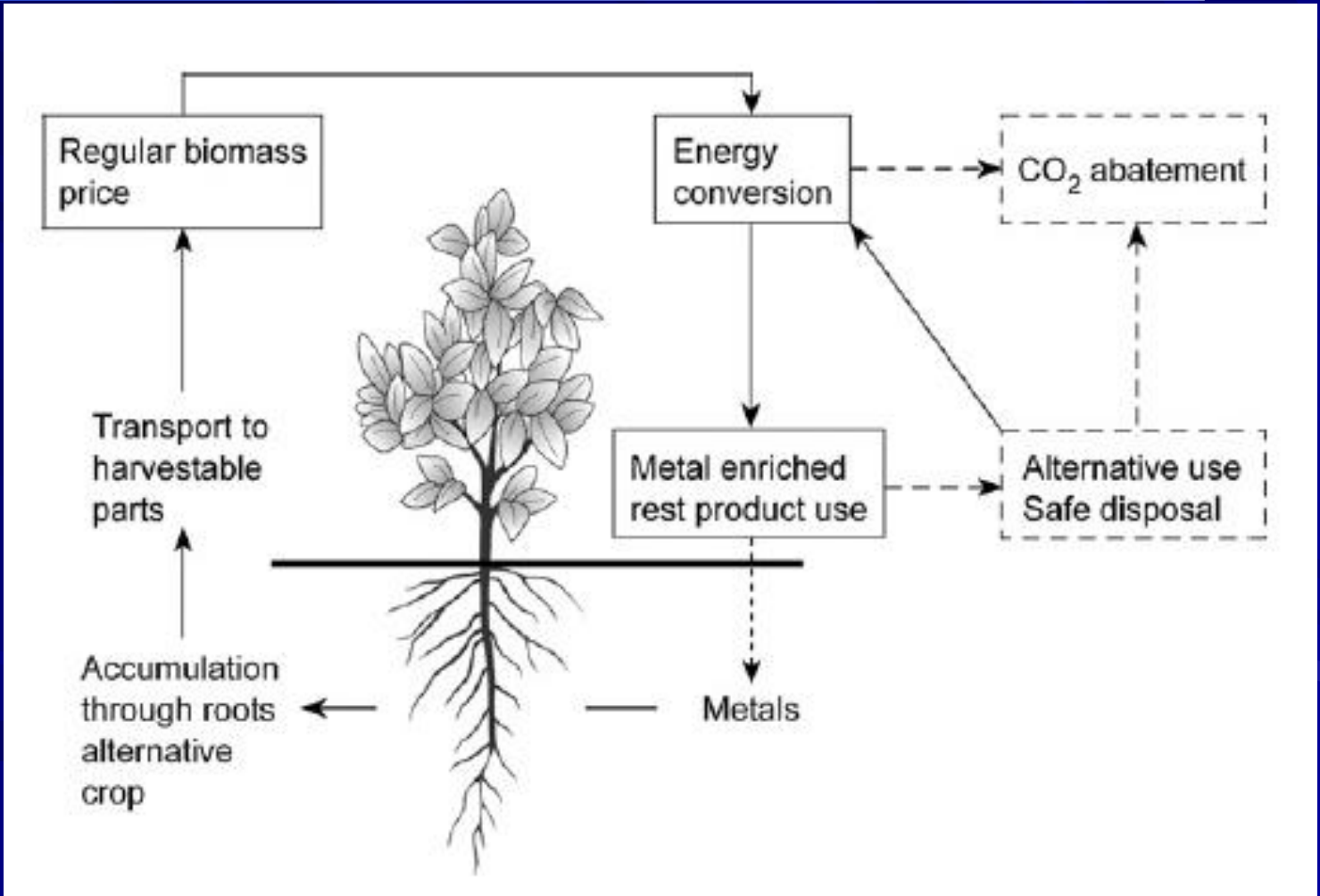
Table 2 Removal of TPH and PAH from contaminated soil (mean values of 3–5 parcels sampling)

Season	Initial TPH (mg kg^{-1})	Final TPH (mg kg^{-1})	Removed TPH (kg ha^{-1})	Initial PAH (μg kg^{-1})	Final PAH (μg kg^{-1})	Removed PAH (kg ha^{-1})
Summer 98						
Alfalfa	3,308	2,405	2,168	6,329	4,960	3.3
Maize	3,235	2,227	2,419	6,680	4,017	12.0
Red clover	3,414	2,227	2,849	8,687	4,373	10.4
Ryegrass	3,751	3,451	721	8,351	6,655	4.1
Land farming	3,322	2,681	1,538	6,846	6,298	1.3
Winter 98–99						
Fescue	3,134	1,144	4,777	6,855	3,960	6.9
Rape	2,403	908	3,588	4,183	1,953	5.4
Rye	3,055	1,208	4,432	5,784	5,037	1.8
Ryegrass	2,687	1,081	3,854	6,258	2,785	8.3
Triticale	4,202	1,930	6,478	6,317	5,794	1.6
Vetch	2,152	765	3,330	5,224	2,916	5.9
Weed	2,440	701	4,173	5,213	1,692	8.5
Land farming	1,716	1,031	1,644	4,428	2,156	5.5
Summer 99						
Alfalfa	1,786	861	2,221	3,673	2,273	3.4
Fescue	2,055	826	2,950	3,861	2,408	3.5
Maize	2,932	561	5,692	4,100	1,927	5.2
Red clover	1,564	677	2,127	4,442	2,720	3.7
Sorghum	2,749	486	5,431	5,230	1,829	8.2
Soya	2,952	1,155	4,313	3,432	1,201	7.0
Weed	2,507	472	4,885	3,467	1,703	6.1
Landfarming	1,009	735	104	2,558	1,634	2.2

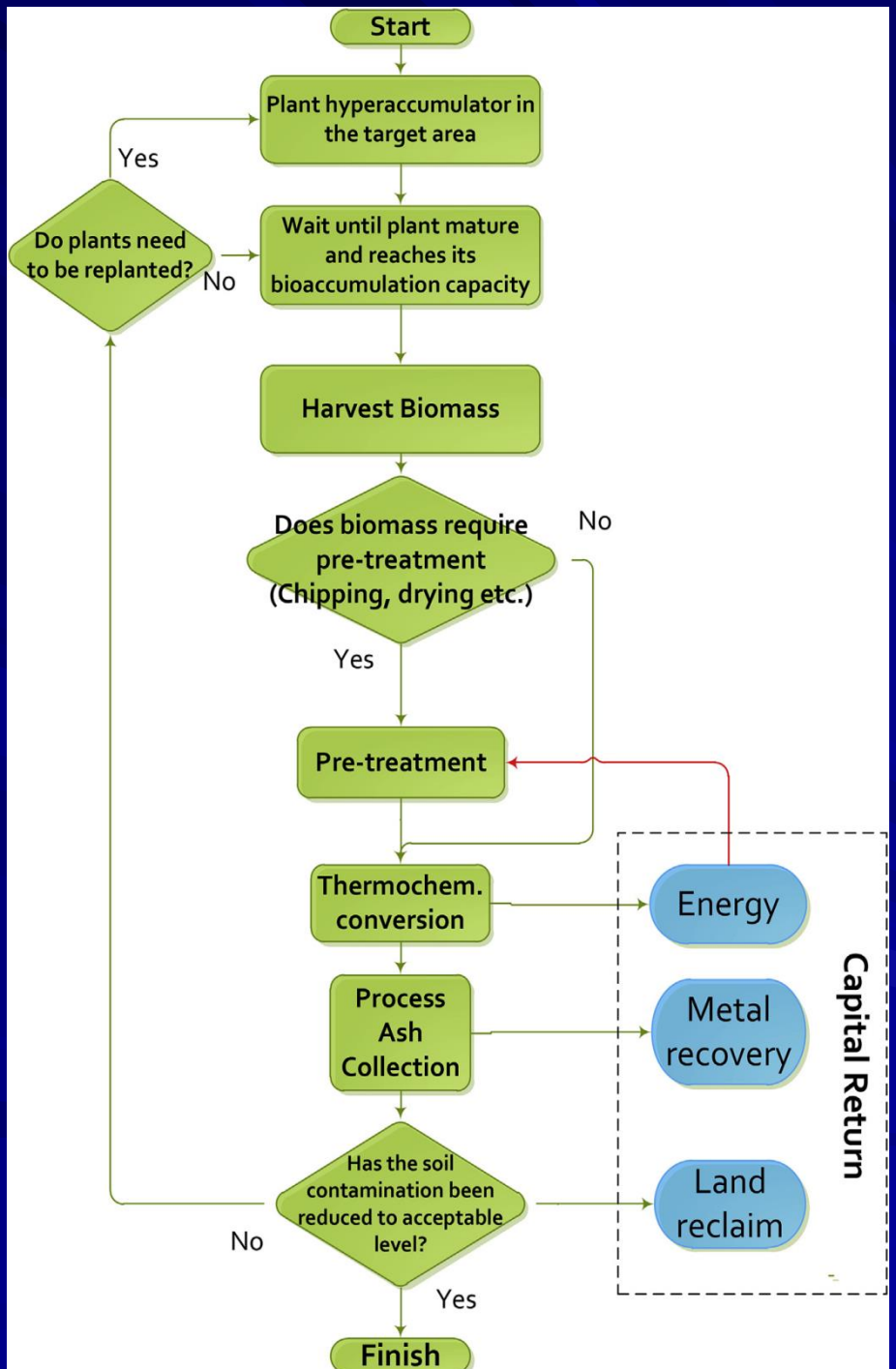


Truu et al. (2015)
Open Biotechnol J
9 (Suppl 1-M9) 85-92

3) Last, but not least challenge:Toward phytomanagement and biorefineries

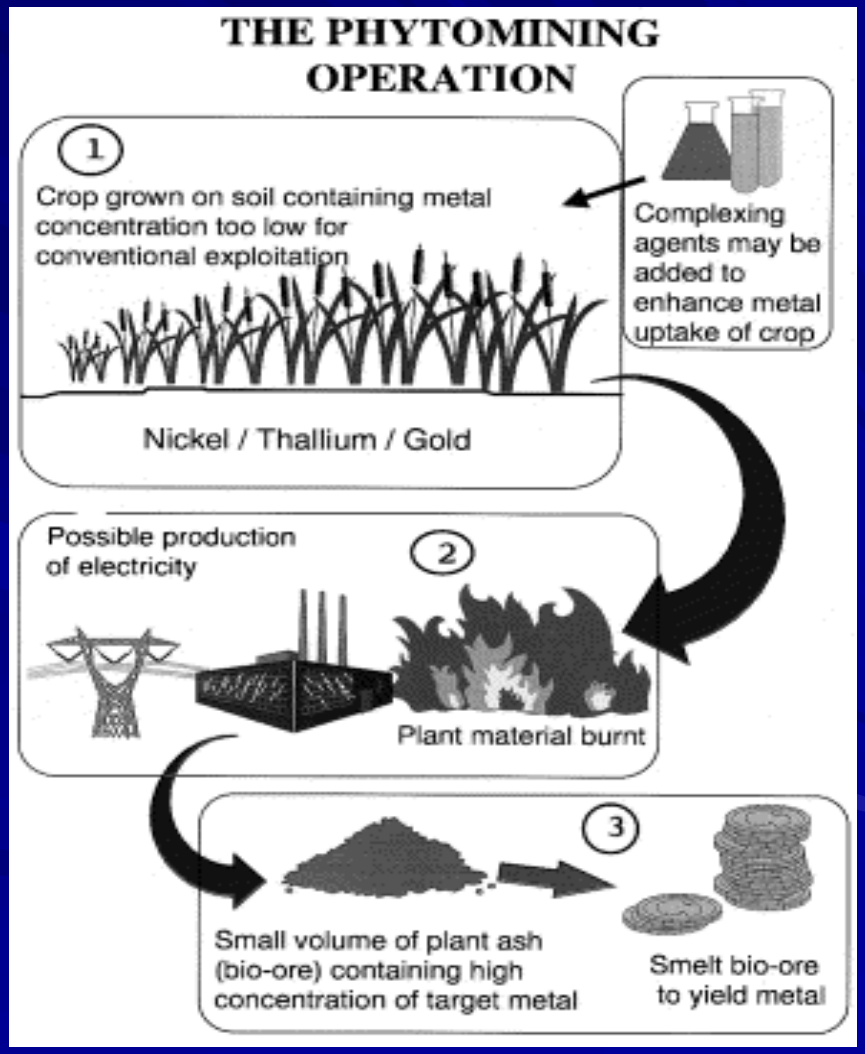


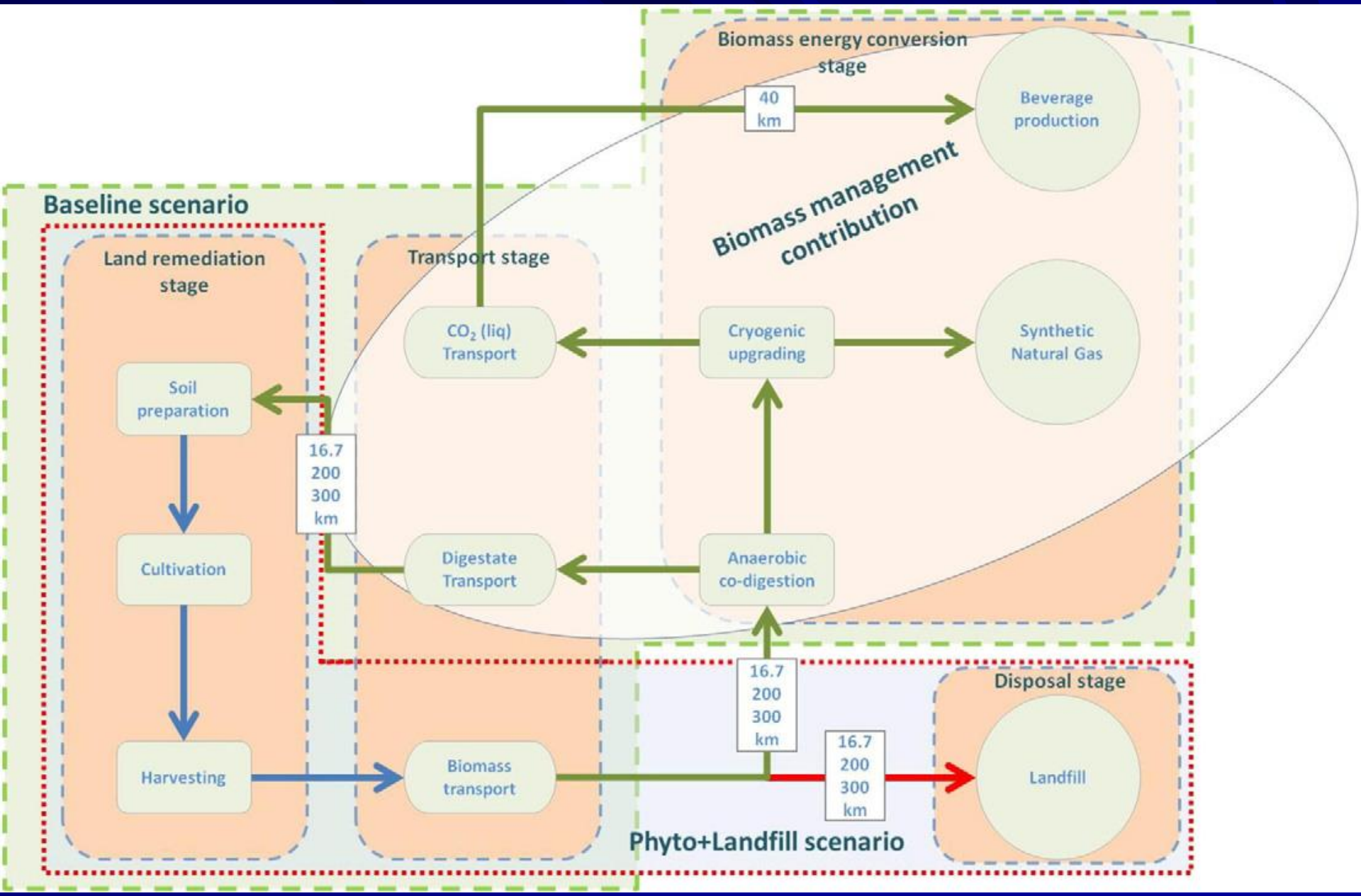
Witters et al. (2012) Biomass Bioen 39:454-469



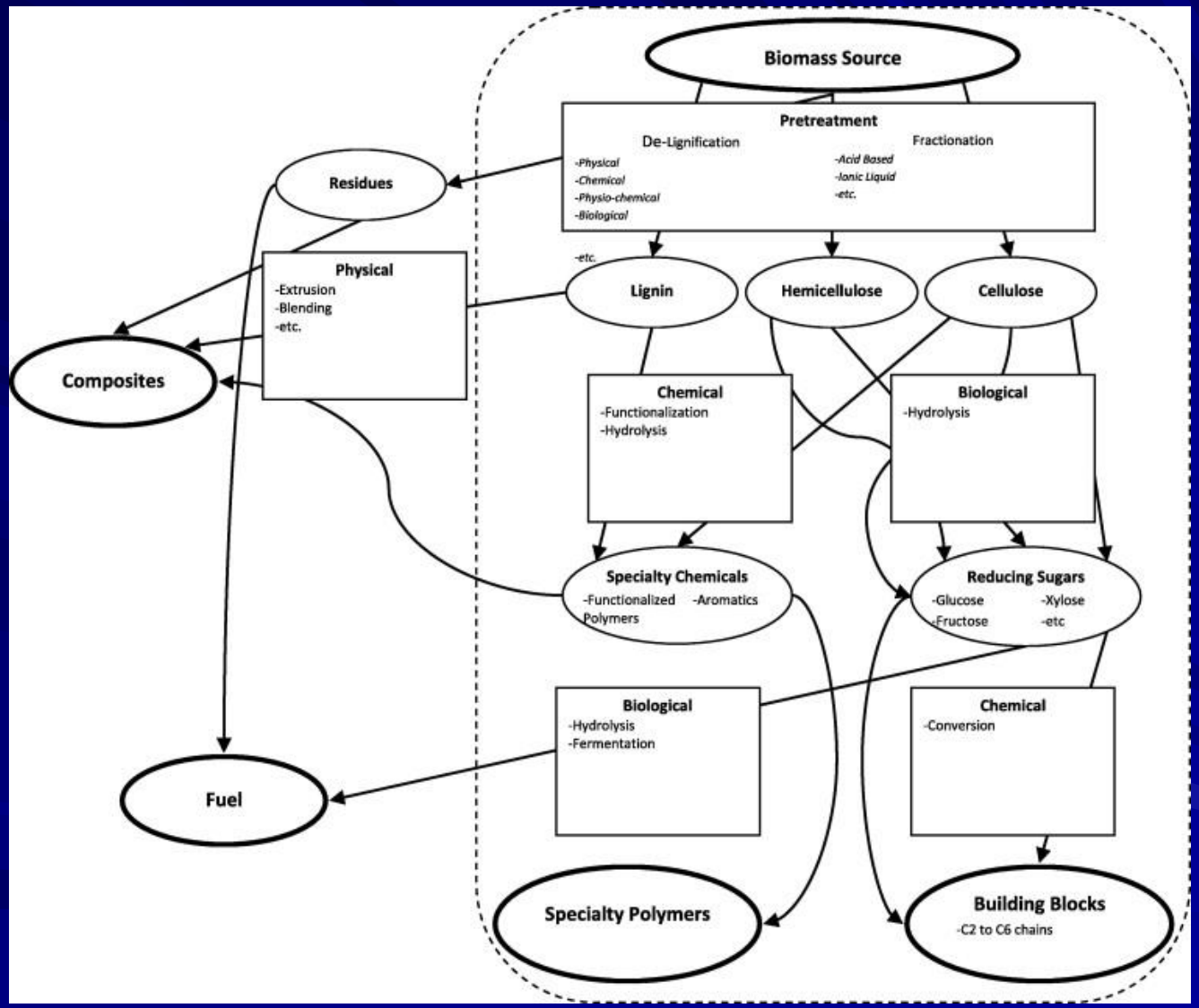
Jiang et al (2016)
Biomass Bioenergy
83:328-339

Phytomining: The use of specific hyperaccumulators for metal recovery





Vigil et al. (2015) Sci Tot Environ 505:844-850



FitzPatrick et al (2010) Biores. Technol. 101:8915

Plants are able to remediate contaminated soils:
It is a fact, even if many challenges remain to be addressed

Thank you for your attention !