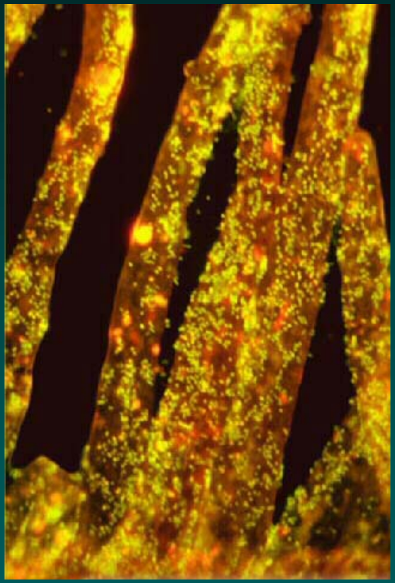
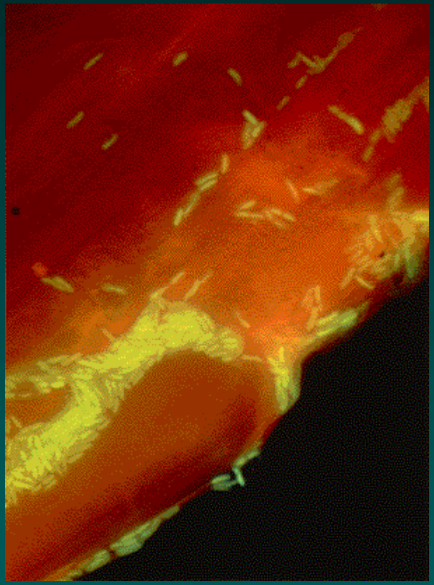
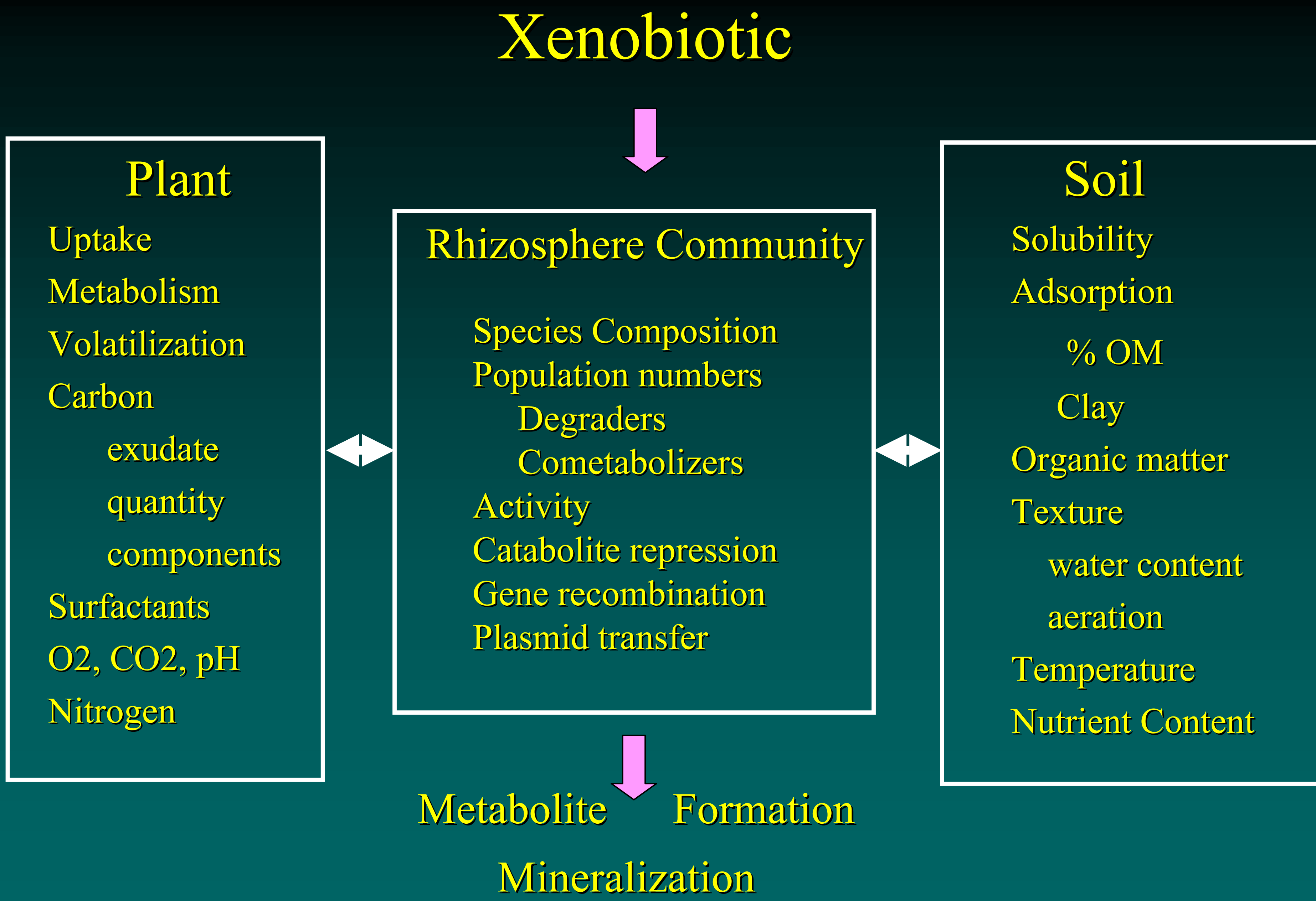
Phytoremediation of PCBs and PAHs with Monoterpene Producing Plants





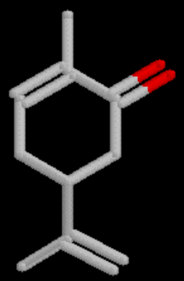
David Crowley, Joong-Wook Park, Haakrho Yi
Department of Environmental Sciences
University of California, Riverside



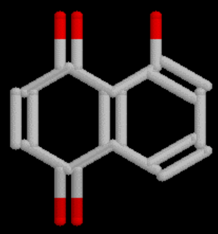
Xenobiotic
Rhizosphere Community
Species Composition
Population numbers
Degraders
Cometabolizers
Activity
Catabolite repression
Gene recombination
Plasmid transfer
Plant
Uptake
Metabolism
Volatilization
Carbon
exudate
quantity
components
Surfactants
O2, CO2, pH
Nitrogen
Soil
Solubility
Adsorption
% OM
Clay
Organic matter
Texture
water content
aeration
Temperature
Nutrient Content
Metabolite Formation
Mineralization

Phytochemical Analogs of Xenobiotic Contaminants

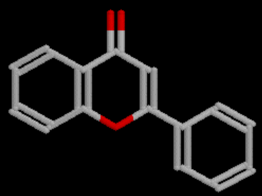
Terpenes:



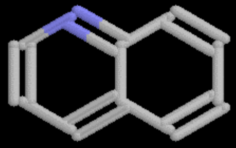
Quinones



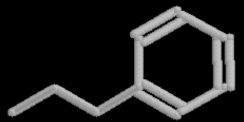
Flavonoids



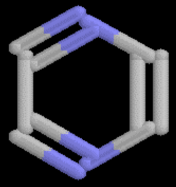
Alkaloids



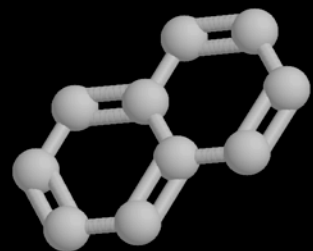
Lignin



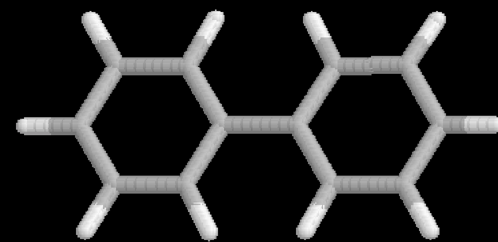
Pyrazines



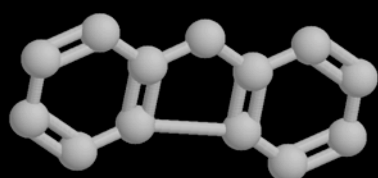
Structures of Common Aromatic Soil Pollutants



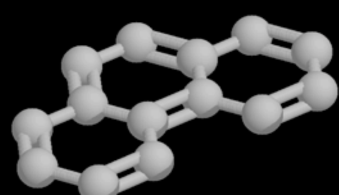
Napthalene



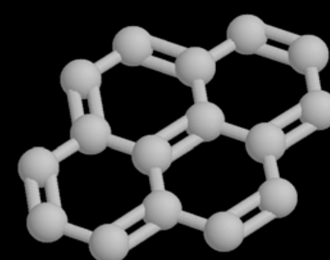
Polychlorinated Biphenyls



Fluorene



Phenanthrene



Pyrene

MDL

Cometabolism of PCBs

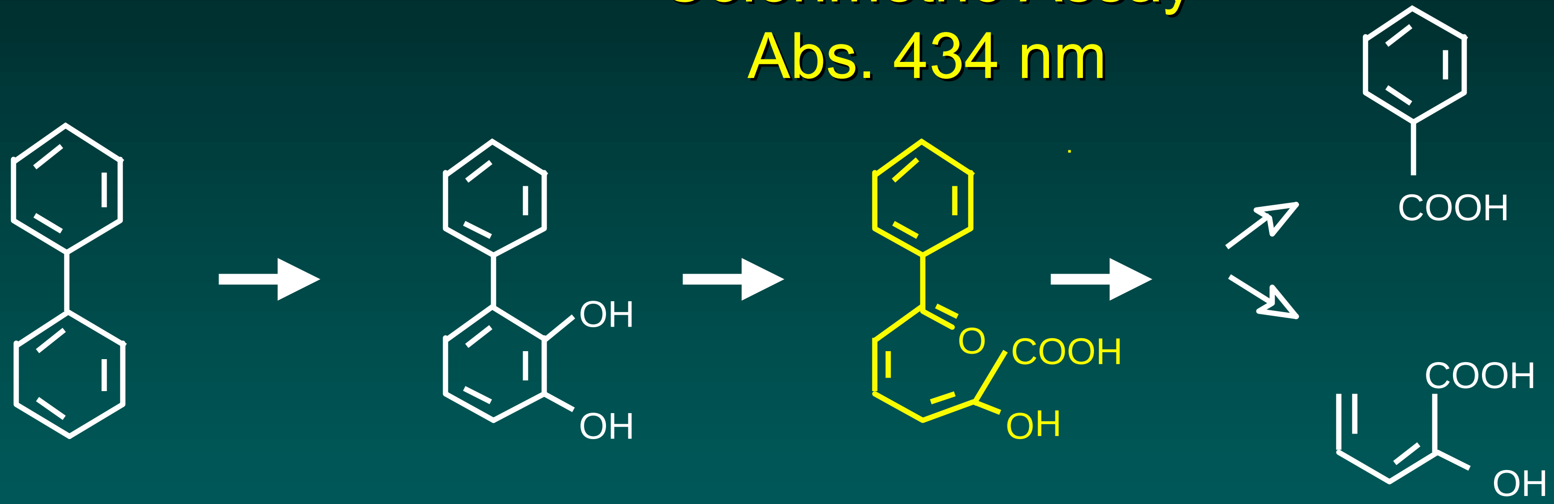
bacteria + biphenyl → growth

bacteria + PCB → no growth

bacteria + biphenyl + PCB → growth + degradation of PCBs

Assay for Cometabolism of PCBs

Colorimetric Assay
Abs. 434 nm



Biphenyl

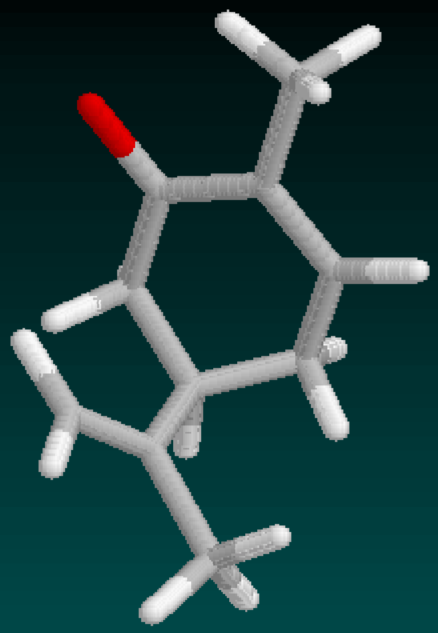
Biphenyl diol

phenylhexadienoate ring fission product

hydrolysis products

Plant Screening Assay

Gilbert and Crowley 1996



Spearmint (*Mentha spicata*)

Active component: carvone

Arthrobacter B1B

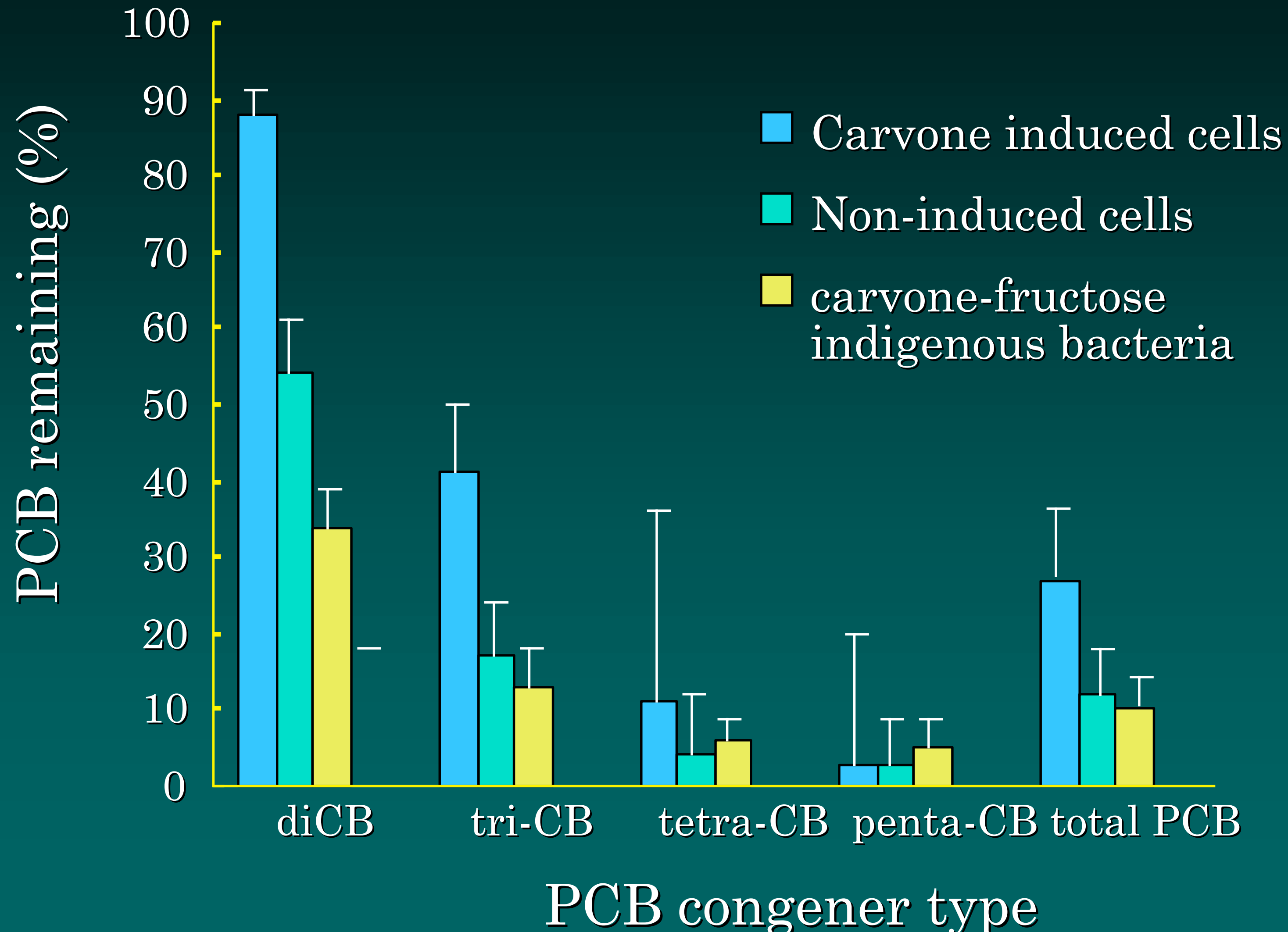
Evidence:

PCB ring oxidation product

chlorobenzoate product formation

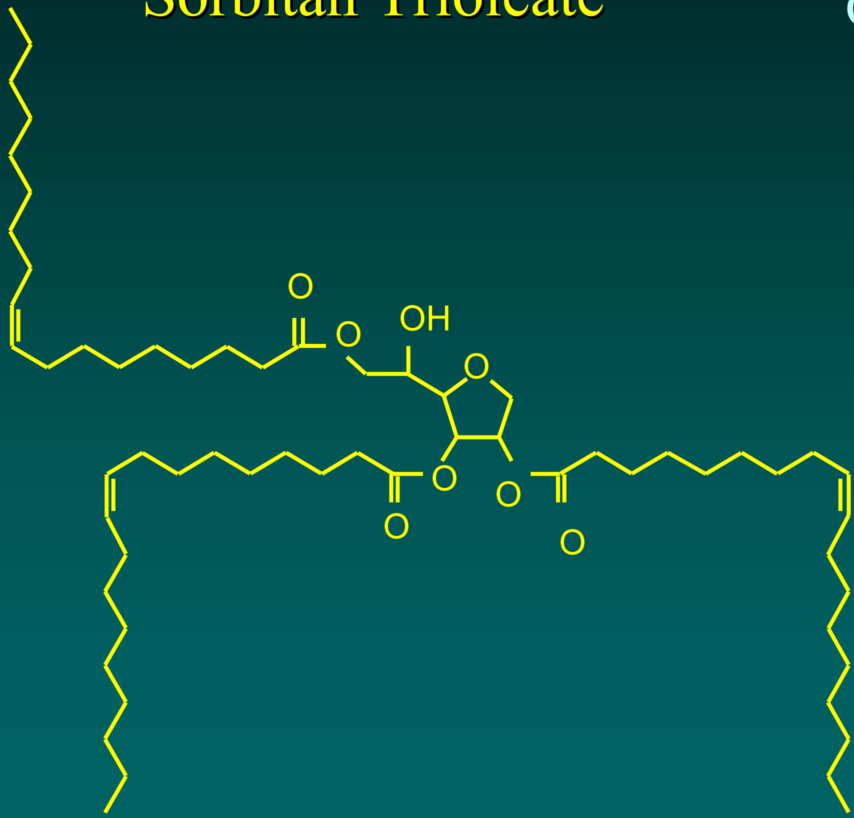
disappearance of Aroclor 1242

Degradation of 100 ppm Aroclor 1242 PCBs in Soil after 9 Weeks After Inoculation With Carvone-Induced Arthrobacter B1B



Development of a Field Application Vector

Sorbitan Trioleate



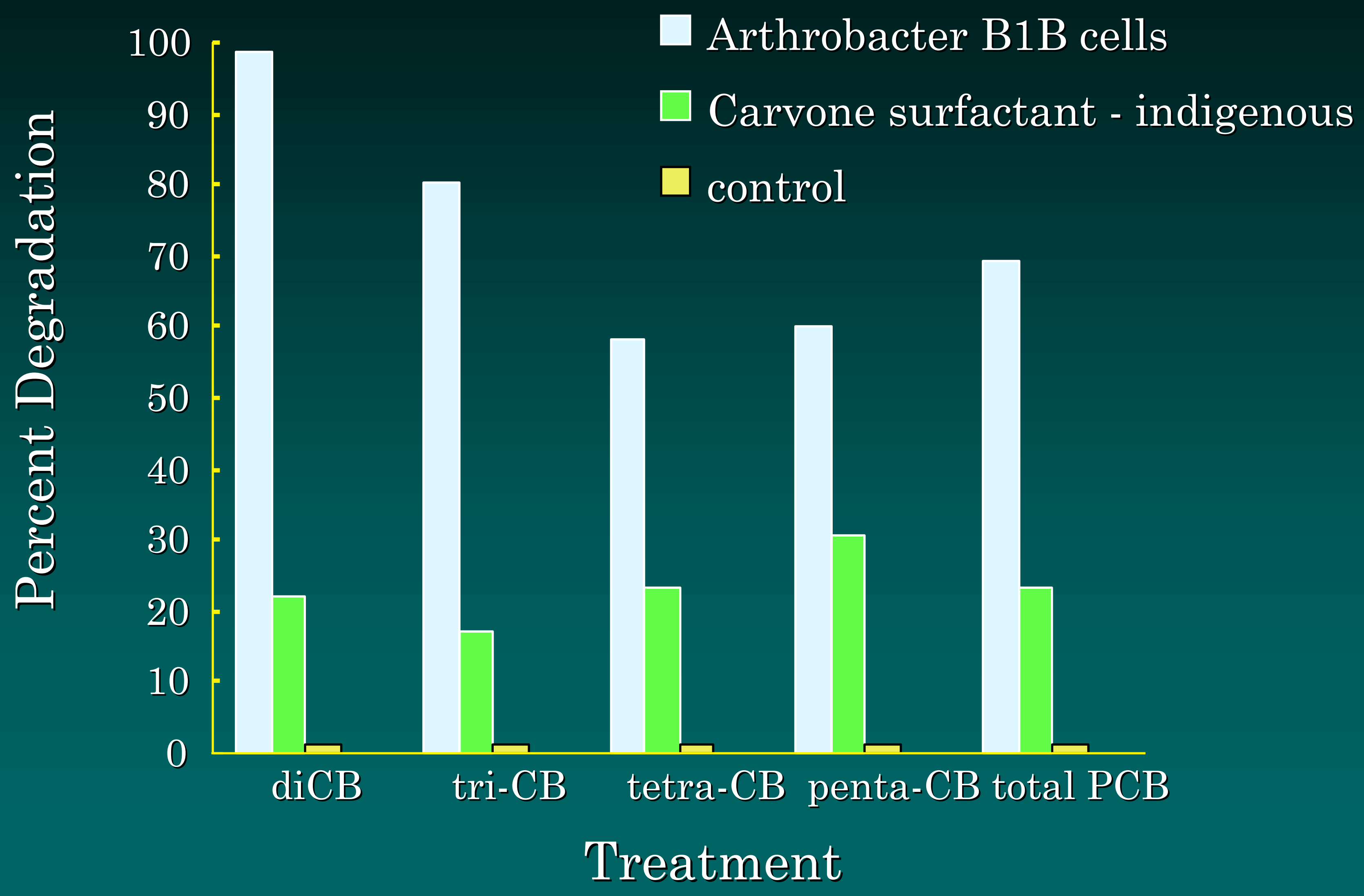
Criteria:

- Nontoxic
- Selective Growth Substrate
- Solubilizes PCB
- Biodegradable

Fatty Acid Composition:

Oleic acid	**74%**
Linoleic acid	**7%**
Linolenic acid	**2%**
Palmitoleic acid	**7%**
Palmitic acid	10%

Degradation of Aroclor 1242 PCBs after 9 Weeks of Repeated Inoculation Using Carvone-Surfactant Grown Cells



Induction of PCB degradation by Carvone and Related Compounds

TABLE 3. Ring fission product formation induced by carvone and structurally similar compounds

Compound[a]	Product formation rate[b]	Significance[c]	Plant source[d]
(1) *p*-Cymene	1,471 ± 343	a	Widely distributed
(2) Isoprene	928 ± 52	b	Widely distributed
(3) (*S*)-(+)-Carvone	860 ± 117	bc	Dill seed and caraway seed
(4) (*R*)-(−)-Carvone	845 ± 187	bc	Spearmint
(5) (*S*)-(−)-Limonene	821 ± 168	bc	Pine needle oil
(6) (*R*)-(+)-Limonene	807 ± 103	bc	Citrus, juniper, and dill seed
(7) Biphenyl	706 ± 72	c	None
(8) Carvacrol	509 ± 19	d	Oregano
(9) Cumene	280 ± 110	e	None
(10) *p*-Xylene	188 ± 80	ef	None
(11) Toluene	138 ± 23	ef	None
(12) *trans*-Cinnamic acid	87 ± 3	f	Storax
(13) Thymol	70 ± 10	f	Thyme
(14) Benzene	61 ± 13	f	None
Fructose only (control)	73 ± 48	f	NA[e]

[a] Numbers correspond to those for structures in Fig. 2.
[b] Values are nanograms of product per milligram of protein per hour.
[c] Rates of compounds marked by same letter are not significantly different ($P < 0.05$; statistical analysis by Fisher's least significant difference test).
[d] Major plant source according to Buckingham (5).
[e] NA, not applicable.

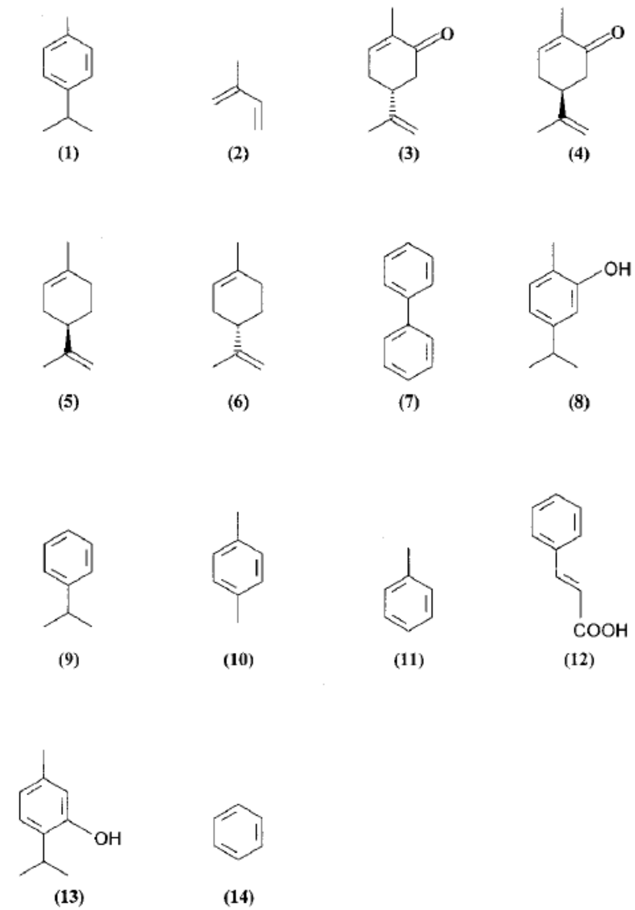
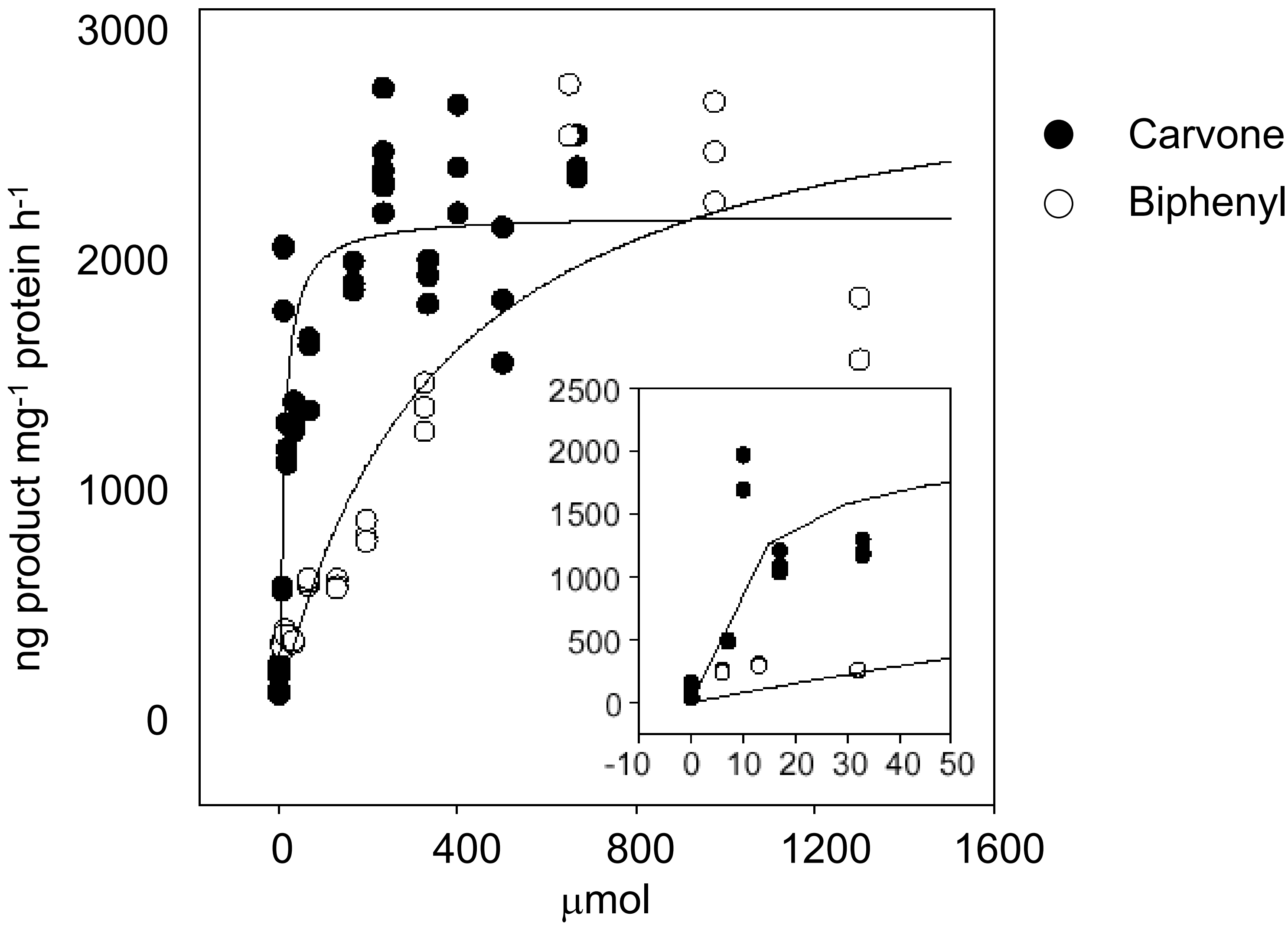
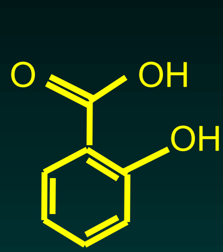


FIG. 2. Structures of compounds used in this study.

PCB ring fission product formation by *Arthrobacter* B1B following enzyme induction by carvone or biphenyl. (Gilbert and Crowley 1998)

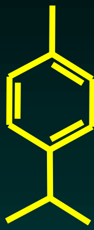




Salicylate

cumene

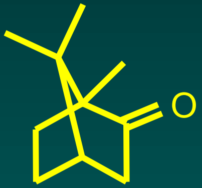
limonene



cymene

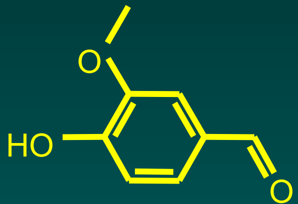
carvone

cineole

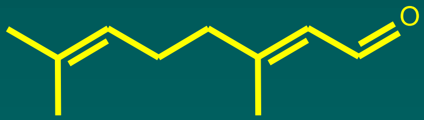


camphor

pinene

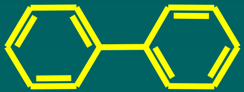


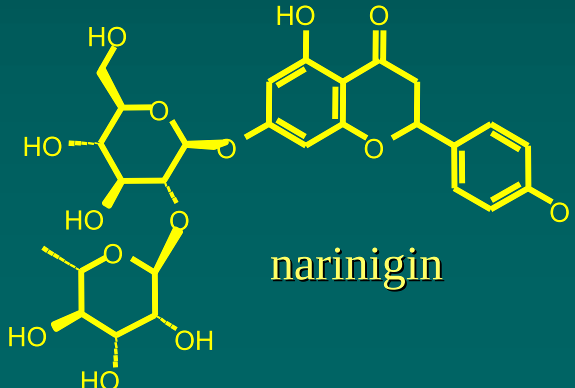
vanillin



citral

biphenyl



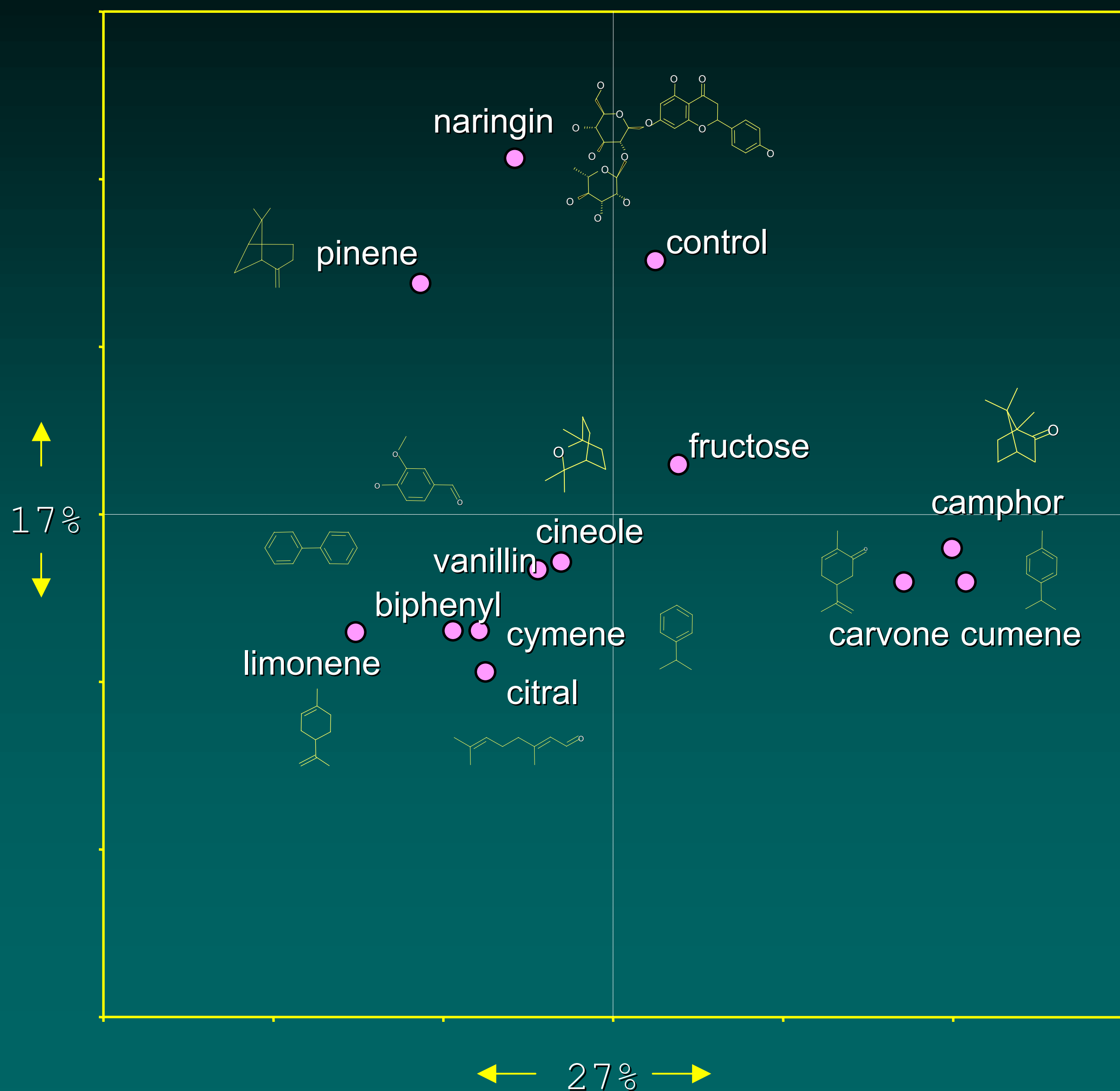


narinigin

BRDU Labeled 16S rDNA Profile of Terpene EnrichedSoil

Biphenyl Vanillin Citral Pinene Limonene Naringin Cumene Cymene Cineole Carvone Camphor Fructose Control

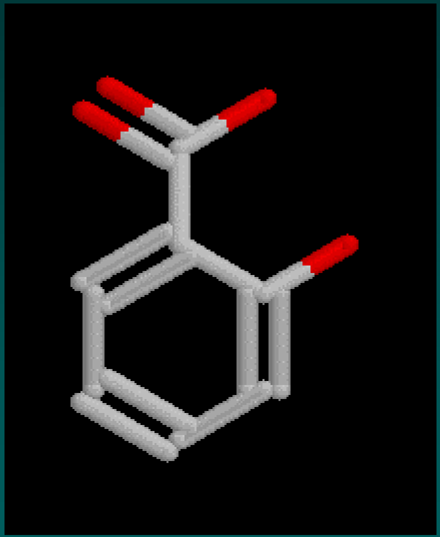
Correspondence Analysis of BRDU Labeled 16S rDNA from Terpene Enriched Soils



Salicylate: Plant Signal Compound, Siderophore, and Inducer of Xenobiotic Degradation Enzymes

Toluene dioxygenase substrates:

- Benzene, toluene, xylene (BTEX)
- Trichloroethylene (TCE)
- Trinitrotoluene (TNT)
- Naphthalene, benzopyrene (PAH)
- Polychlorinated biphenyls (PCB)



Coinoculation with *Arthrobacter* sp. B1B and *Ralstonia eutrophus* H850

PCB congener	*Arthrobacter*	*Ralstonia* coinoculation
2,3,6,2'	34 ± 5	45 ± 6
2,5,2',5'	29 ± 3	56 ± 7
2,4,2',5'	27 ± 3	49 ± 6
2,4,2',4', 2,4,5,2'	30 ± 5	44 ± 6
2,3,2',3'	30 ± 6	51 ± 6
2,3,5,2',4', 2,4,5,2',5	13 ± 4	25 ± 4

Summary

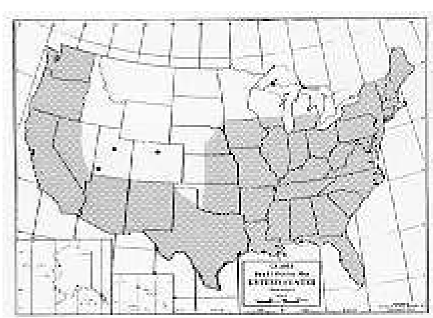
- Gram positive bacteria induced by monoterpenes.
- Gram negative bacteria induced by salicylic acid.
- Many indigenous PCB degraders can be enriched by growth on these substances.

Common terpene producing plant species

Origanum majorana
O. vulgare
Thymus zygis
Curcuma longa
Eucalyptus species
Vitex negundo
Ligusticum porteri
Andropogon sp.
O. syriacum
Salvia cabulica
T. piperella
Carum copticum
Tanacetum parthenium
Acanthopanax trifoliatus
Ocimum basilicum
Chenopodium sp.

Chenopodium ambrosioides

Alpha-pinene, Aritasone, Ascaridole, Ascorbic-acid, Beta-carotene, Butyric-acid, Calcium, D-camphor, EO, Ferulic-acid, Geraniol, L-pinocarvone, Leucine, Limonene, Malic-acid, Menthadiene, Methyl-salicylate, Myrcene, Niacin, P-cymene, P-cymol, Phosphorus, Safrole, Saponins, Spinasterol, Tartaric-acid, Terpinene, Terpinyl-acetate, Terpinyl-salicylate, Thiamin, Triacontyl-alcohol, Trimethylamine, Urease, Vanillic-acid



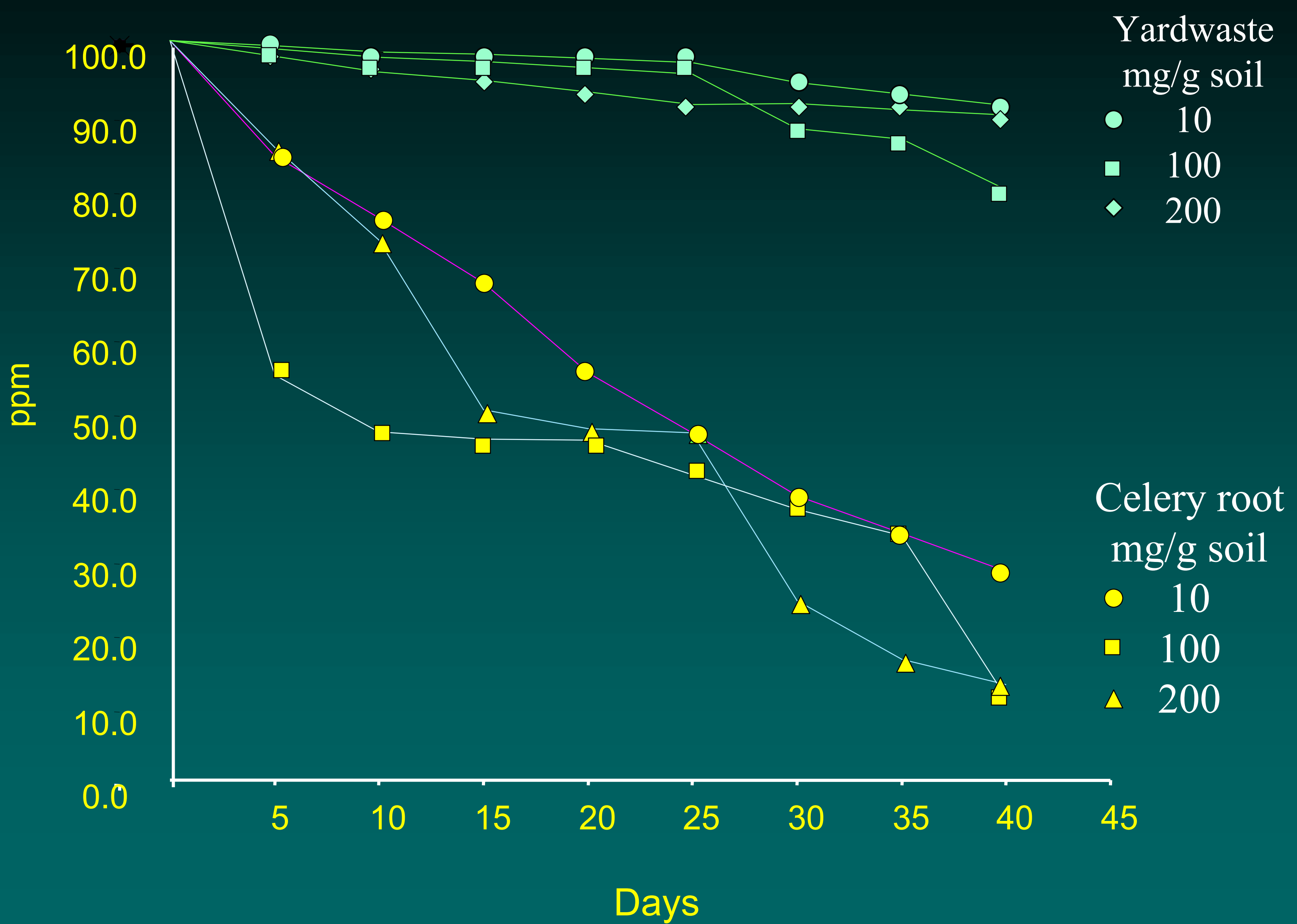
Phytoremediation of Pyrene using Celery Root

Root essential oil:

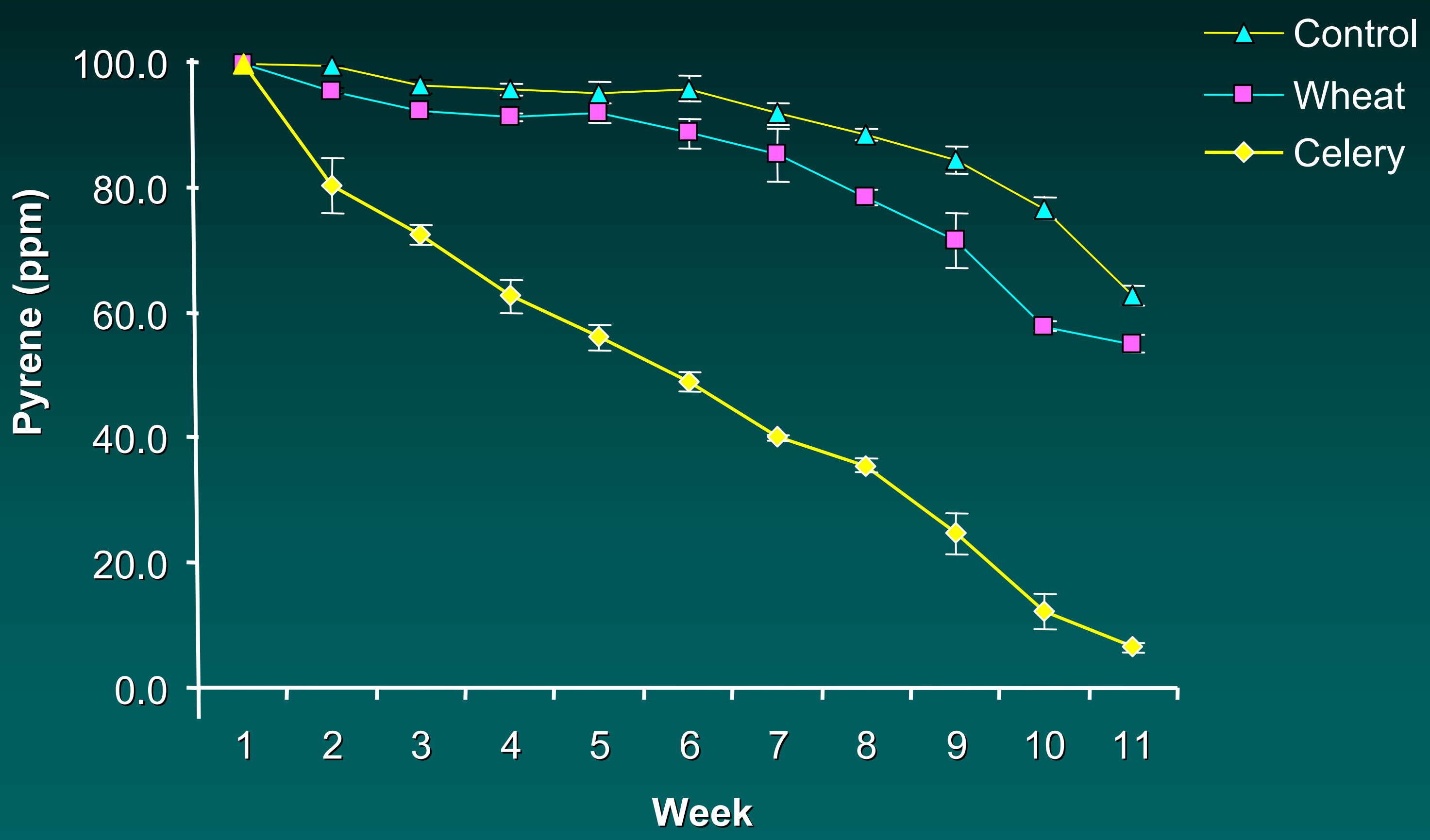
	ppm
β pinene	15,000
carvone	5000
dihydro-carvone	5000
P-cymene	31,000
limonene	117.000
myrcene	18,000
terpenoline	33,000
trans-ocimine	290,000
cis-ocimine	68,000

Duke, J. A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.

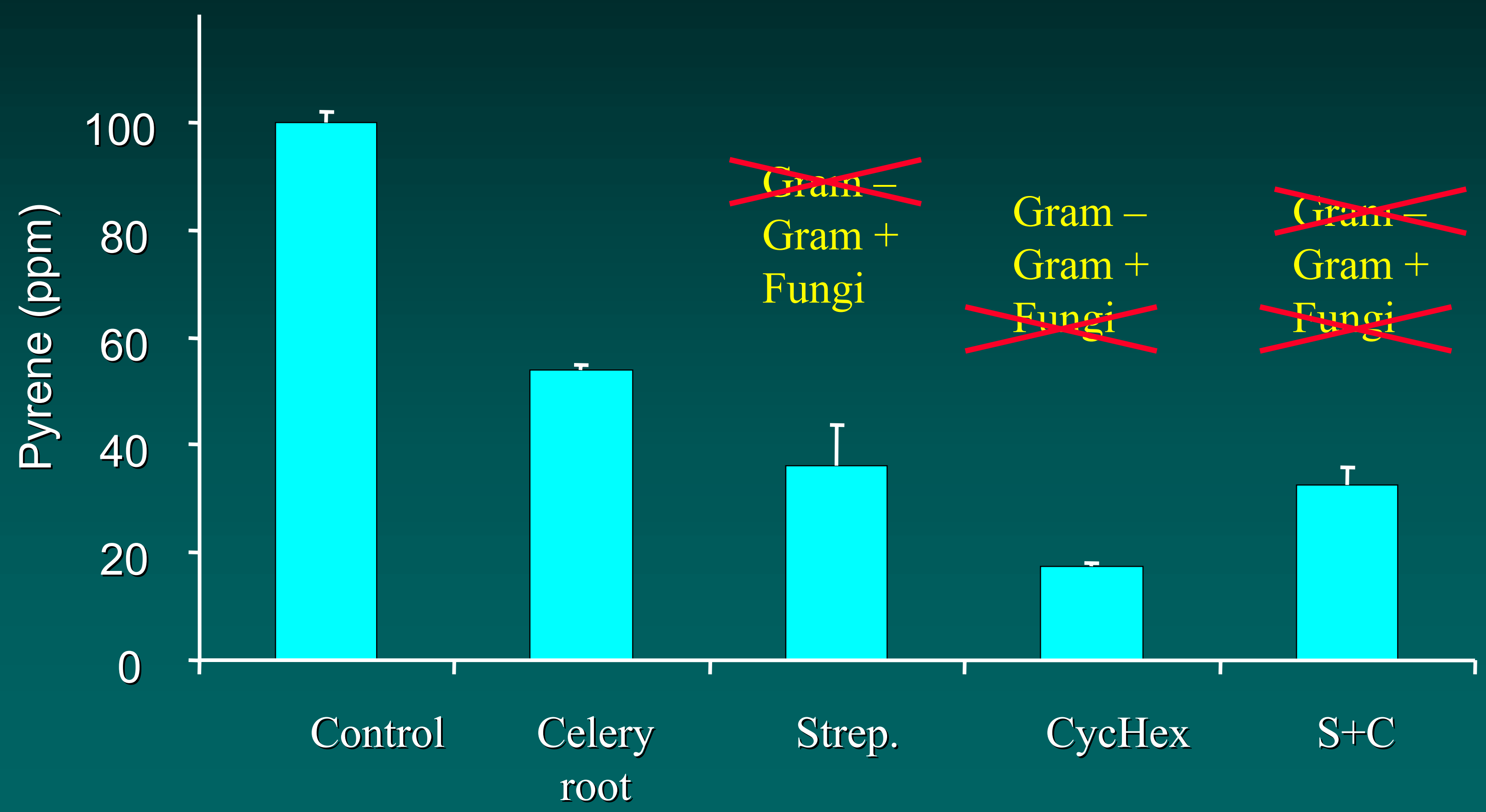
Degradation of pyrene in soil amended with celery root



Degradation of pyrene in the rhizosphere of wheat and celery plants



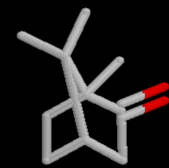
Selective inhibitors suggest Gram + bacteria are predominant pyrene degraders induced by celery root.



What enzymes are induced by monoterpenes?

Biodegradation of Camphor: Role of P450CAM

camphor



↓ camphor 5-monooxygenase (cytochrome P450 cam)

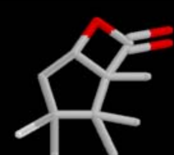
5 exo-hydroxycamphor

↓ 5-exo-hydroxycamphor dehydrogenase

2,5 diketocamphane

↓ camphor 1,2-monooxygenase (Baeyer-Villiger monooxygenase)

5-Oxo-1,2-campholide



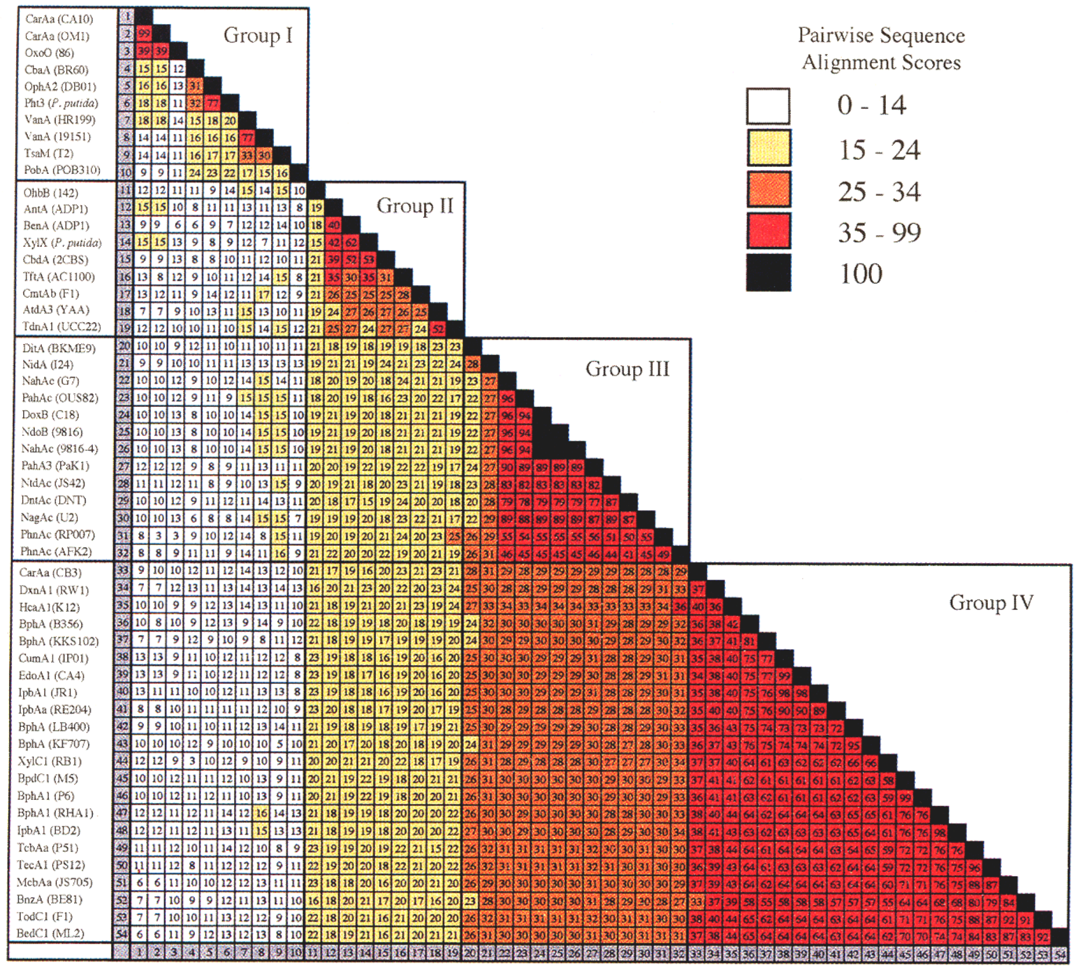
Pairwise sequence alignment scores for α subunits of ring hydroxylating oxygenases (Nam et al 2000)

Analine

Toluene

PAH / Terpene

Biphenyl



Amino Acid Sequence Alignments of Riske Iron Center Regions of Group 3 PAH and Terpine Dioxygenases

DitA	96	VTLNVOPHRGMRISTADCGNTQ-IHKOIYHGWAFR	119···201	IVNANWKTAGEQSAADGFHT-LTLHRWLGE	229
NidA	83	GHLNAORHRGMQVCRAEMGNAS-HFROPYHGWTYS	116···198	VVDANWKLGADNFVGDAYHT-MMTHRSMVE	226
NahAc G4	76	AFLNVORHRGKTLVNAEAGNAK-GFVOSYHGWGFG	109···190	VIKANWKAPAENFVGDAYHV-GWTHASSLR	218
PahAc	76	AFLNVORHRGKTLVNAEAGNAK-GFVOSYHGWGFG	109···190	VIKANWKAPAENFVGDAYHV-GWTHASSLC	218
DoxB	76	AFLNVORHRGKTLVSVEAGNAK-GFVOSYHGWGFG	109···190	VIKANWKAPAENFVGDAYHV-GWTHASSLR	218
NdoB	76	AFLNVORHRGKTLVSVEAGNAK-GFVOSYHGWGFG	109···190	VIKANWKAPAENFVGDAYHV-GWTHASSLR	218
NahAc 9816-4	76	AFLNVORHRGKTLVSVEAGNAK-GFVOSYHGWGFG	109···190	VIKANWKAPAENFVGDAYHV-GWTHASSLR	218
PahA3	76	AFLNVORHRGKTLVHAEAGNAK-GFVOSYHGWGFG	109···190	IIKANWKAPAENFTGDAYHV-GWTHASSLR	218
NtdAc	74	AFLNVORHRGKTLVHTEAGNAK-GFVOGYHGWGYG	107···188	VVKANWKPFAENFVGDTYHV-GWTHAAALR	216
DntAc	79	AFLNVORHRGKTIVDAEAGNAK-GFVOGYHGWGYG	112···193	VVKGNWKVFAENFVGDTYHI-GWTHASILR	221
NagAc	74	AFLNVORHRGKTLVHAEAGNAK-GFVOSYHGWGFG	107···188	VIKANWKAPAENFVGDAYHV-GWTHASSLR	216
PhnAc RP007	77	AFLNVORHRGARLCAVEAGNAR-GFAONYHGWAYG	110···191	FIEANWKAPSENFVGDAYHV-GWTHASALR	219
PhnAc AFK2	81	AFLNVOTHRGARLVAAEAANAR-AFSOTYHGWSFG	114···194	LLNCNWKTPAENFVGDAYHV-GWTHLASLM	222

Conclusions

- Degradation of xenobiotics in the rhizosphere is likely enhanced by a combination of mechanisms, including: fortuitous enrichment of degraders, growth linked metabolism, and cometabolism.

- Plant signal molecules including terpenes, flavonoids, and salicylate have multiple ecological functions. When released into the rhizosphere, these compounds also have signal functions for inducing cometabolism of organic xenobiotics.

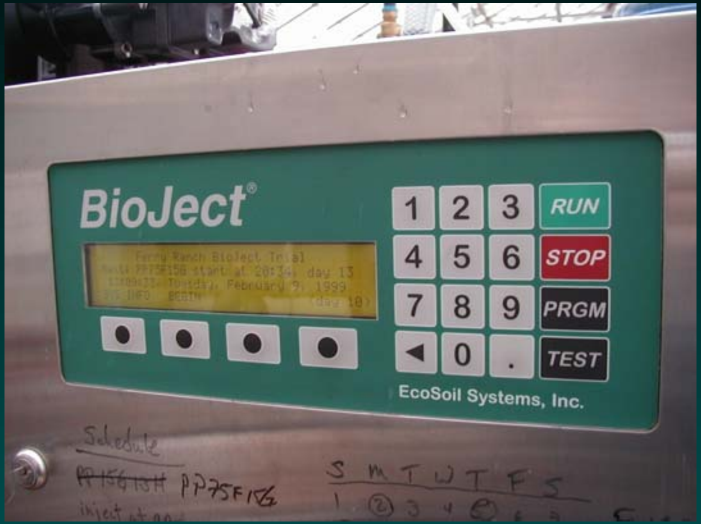
Research Needs

- Identification of specific plants that produce substances that can biostimulate xenobiotic degradation.
- Evaluation of the role of plant, microbial, and earthworm enzymes (oxygenase, cytochrome P450 …) for effectiveness in degrading soil contaminants.
- Studies on the ecology of rhizosphere microbial consortia for enhancing bioremediation.

Acknowledgments

Eric Gilbert
Andrew Singer
Ekawan Luepromchai
Joong Wook Park
Haakrho Yi

Development of Methods for Soil Bioaugmentation



Enzymes for Transformations of Monoterpenes