

Plant-facilitated Reductive Dechlorination of Hexachlorobenzene in Constructed Wetlands

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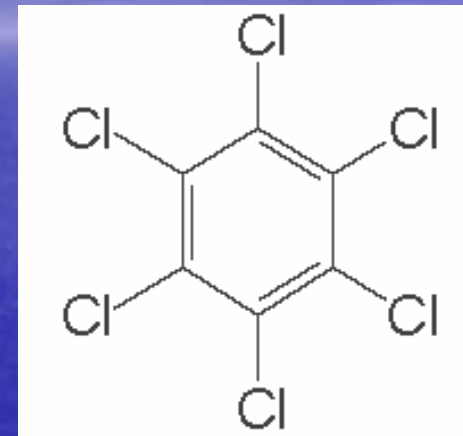
Agenda

- Background
- Experimental Setup and Analysis
- Results
- Conclusions



Background: hexachlorobenzene

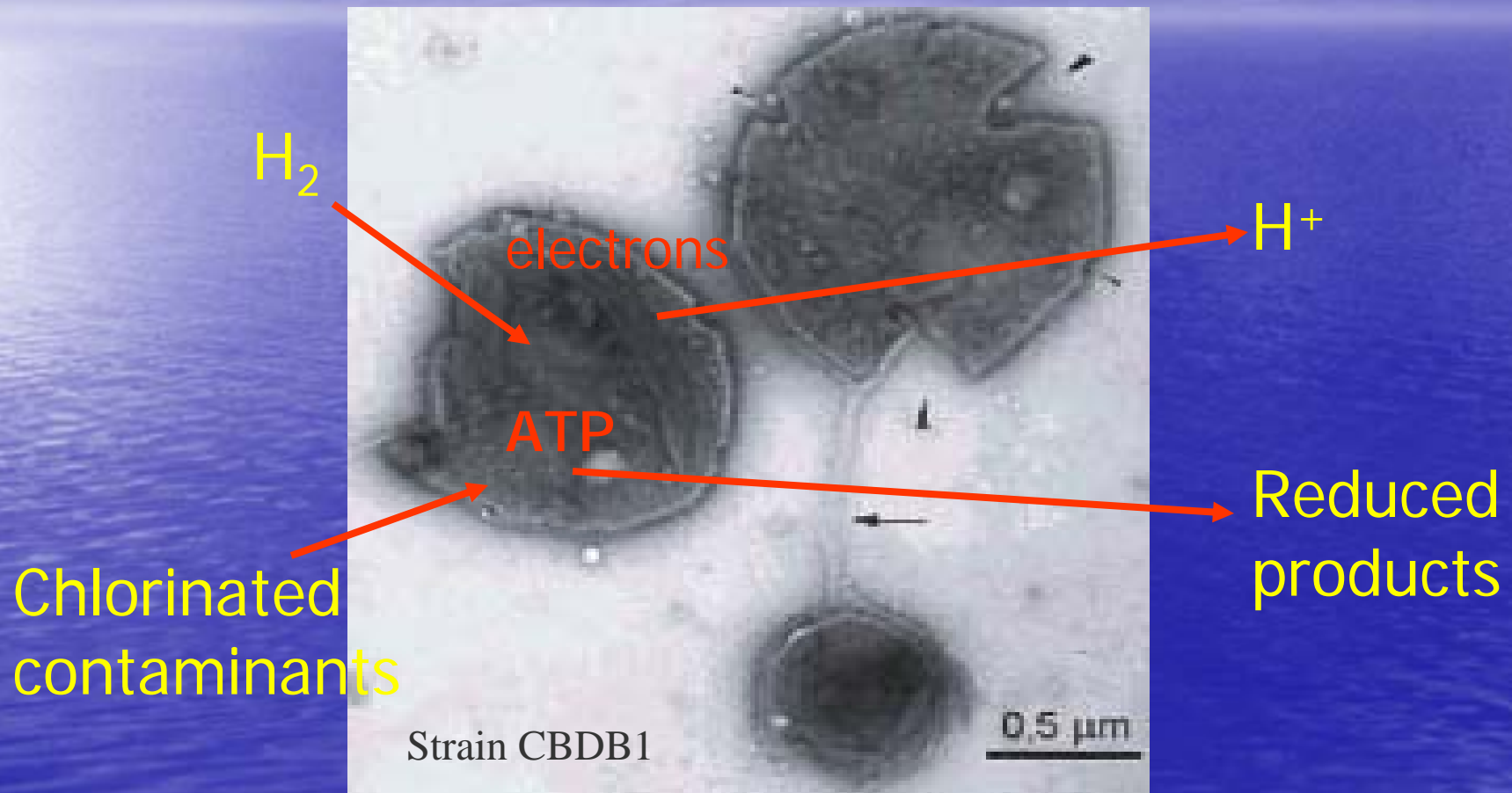
- White crystalline solid
- Used as pesticides, fireworks
- highly oxidized
- Recalcitrant to biological process
- Reductive dechlorination is the only known biological process



Background: Reductive dechlorination

- Carbon and energy source
- Cometabolism
- Dehalorespiration
 - Low G+C Gram positive
 - Proteobacteria
 - Green non-sulfur bacteria
 - *Dehalococcoides*

Background: *Dehalococcoides*



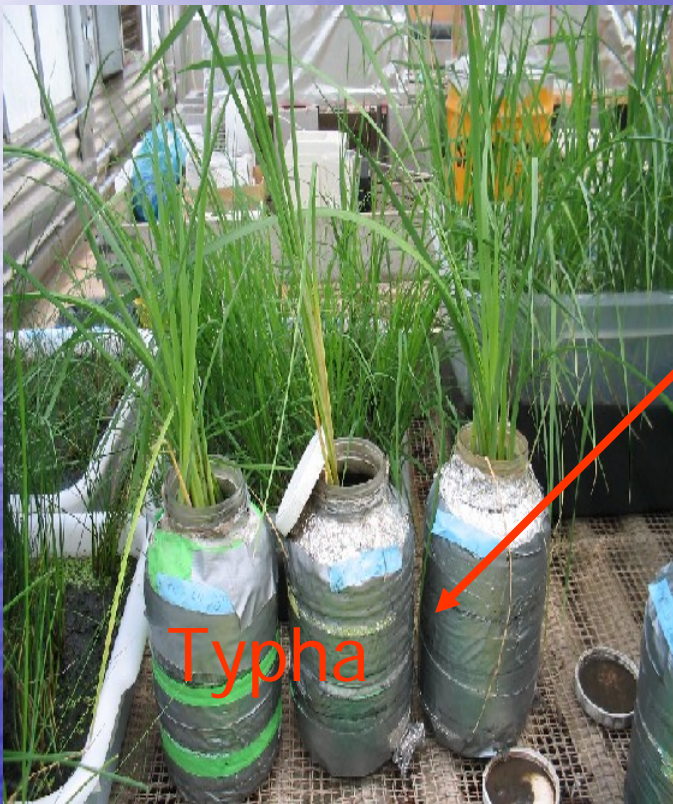
Courtesy to Nature

Background: Phytoremediation

- A technology to use plants to treat contaminated media
- Many advantages over other technologies
- Successful examples
- Less successful examples
- Plant-contaminant-microbes continuum

Experimental Setup

- 4L glass jars
- Artificially aged contaminants in sediments
- Two wetland plants



Materials and Methods-aging

- Prepared 500 ppm HCB in diethyl ether (100mL)
- Added the solution to a 4L jar
- Flushed away the solvents with pure nitrogen
- Added river sediment to about 65% of its volume
- tumbled it for 70 days

Materials and Methods-sampling

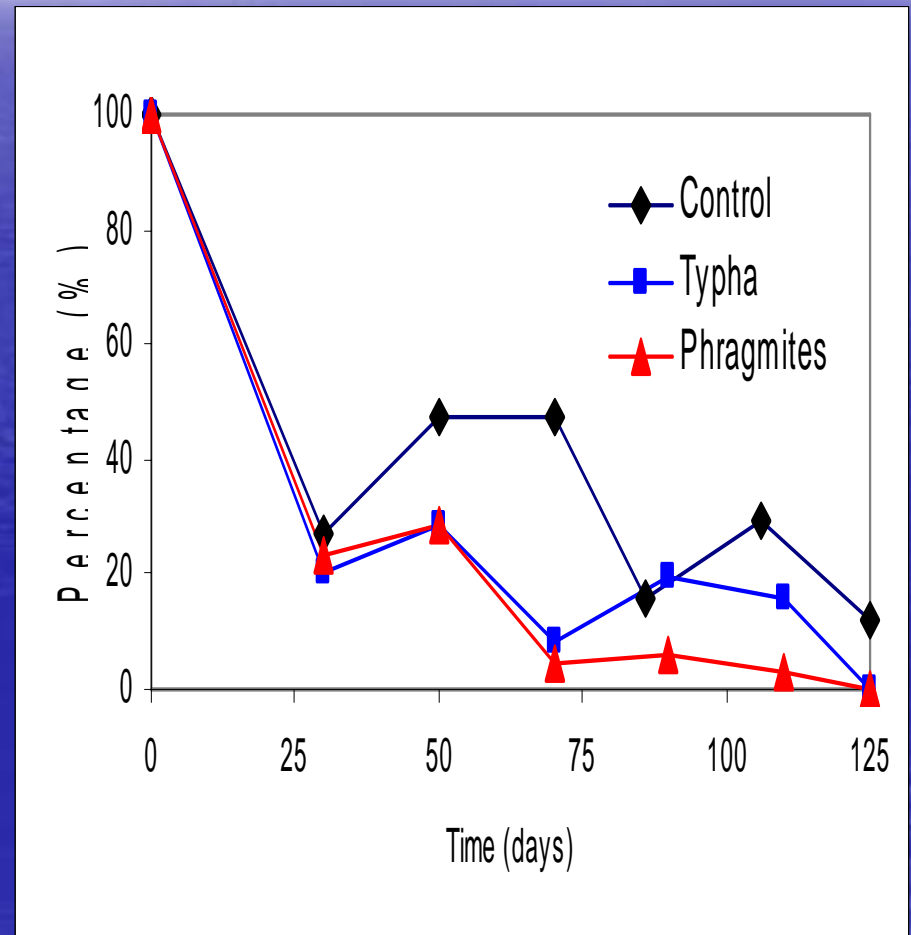
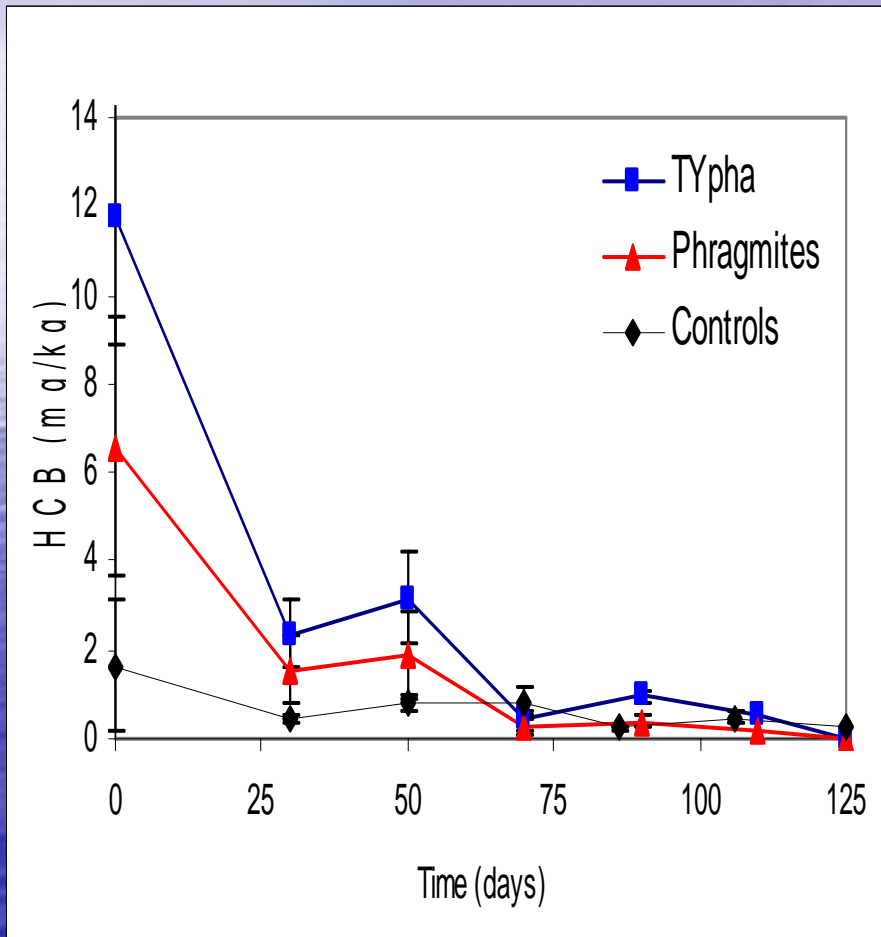


- Sampled every 20 -30 days
 - 10-15g for HCB and metabolites analysis
 - 1-2g for microbial analysis
- 2 planted reactors sacrificed at day 125

Chemical analysis

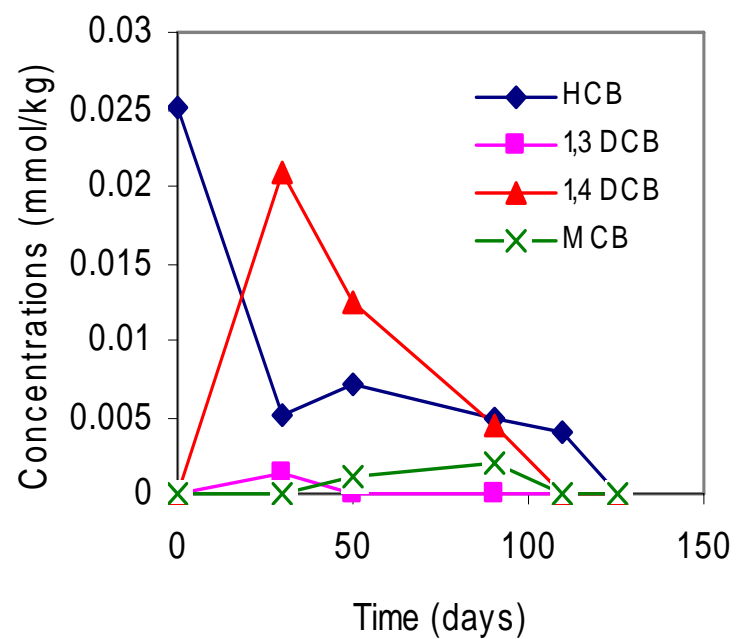
- 10 mL hexane/acetone (50%) added
- Mixed 24 hours
- Centrifuged at 3000rpm for 20 minutes
- Taken 1 mL supernatant for analysis with GC/MS
- 10 uL internal standards added

Results: HCB removal

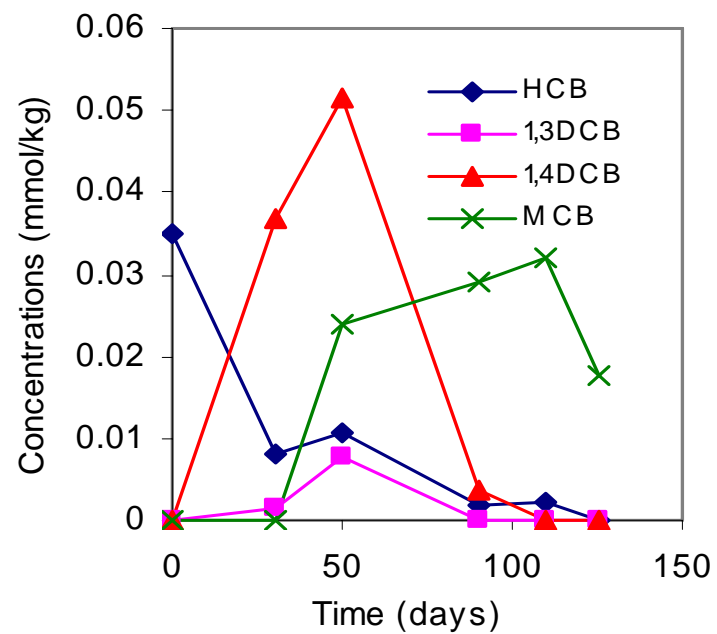


Results-metabolites

Typha



Phragmites



Microbial analysis-DNA extraction

- Sediments
 - Powersoil[®] DNA extraction Kit
- Roots
 - Washed with DI water and PBS washing buffer
 - Lyophilized at -50°C for more than 60h
 - Pulverized under liquid nitrogen
 - Extracted DNA
 - Stored in 100 uL autoclaved DI water

Real-time PCR: Primers

- Performed in iCycle PCR Detection System

Primer	Base pairs	Size
U341F* B907R	CCTACGGGAGGCGAGCAG CCGTCAATTCMTTTRAGTTT	585
DHC774F# DHC1212R	GGGAGTATCGACCCTCTC GGATTAGCTCCAGTTCACACTG	438

* Casemayer et al. 2000.

Hendrickson et al., 2002.

RT-PCR: Temperature programs

- Reaction conditions

	Bacteria	<i>Dehalococcoides</i>
Denaturation(x1)	95°C 3 min	95°C 3 min
Reaction (x40)	94°C 30 s	94°C 30 s
	55°C 1 min	60°C 1 min
	72°C 1 min	72°C 1 min
Extension	72°C 6 min	72°C 6 min
Melting curve (x80)	55°C 10s	60°C 10s

Real Time -PCR: reagents

- RT-PCR reagents (25ul reactions)

SYBR green PCR mastermix	12.5uL
50 nM forward primers	0.15uL
50 nM reverse primers	0.15uL
DNA template	1.5ul
Molecular grade DI water	10.7uL

Real time PCR: standards

- Serially dilute known amount DNA

- Standards for bacteria

E. coli genomic DNA extracted from *E. coli* cells

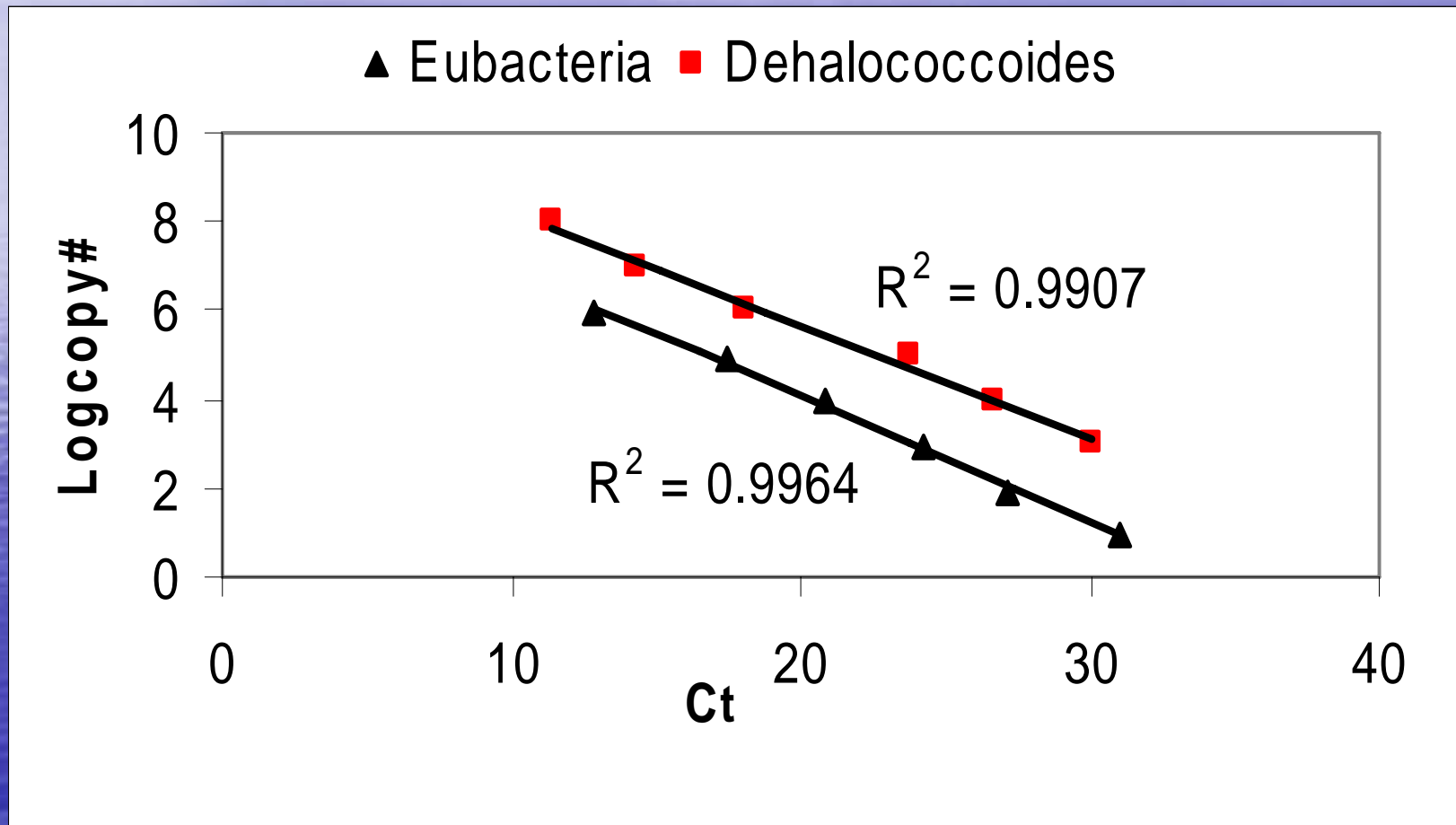
- Standards for *Dehalococcoides*

E. coli plasmid DNA with *Dehalococcoides* 16S rDNA insertion

- DNA copy number calculation

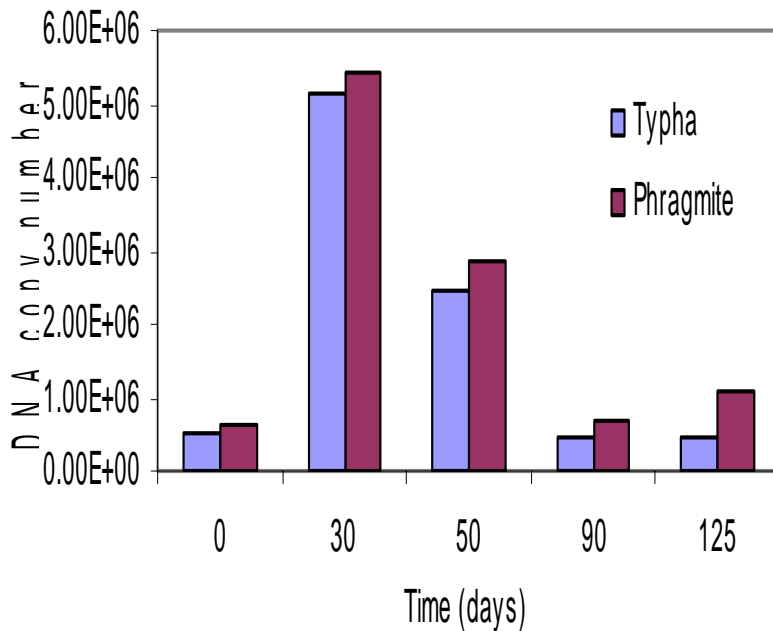
$$\text{DNA\#} = \frac{\text{mass} \times (6.023 \times 10^{23})}{660\text{g/mol} \times \text{length}}$$

Results: RT-PCR standards

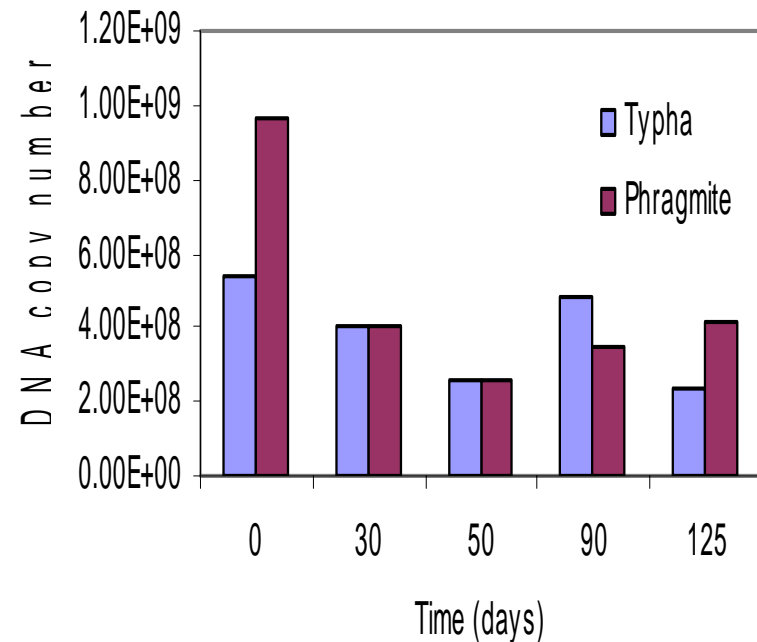


Results: Microorganisms in the bulk sediment over time

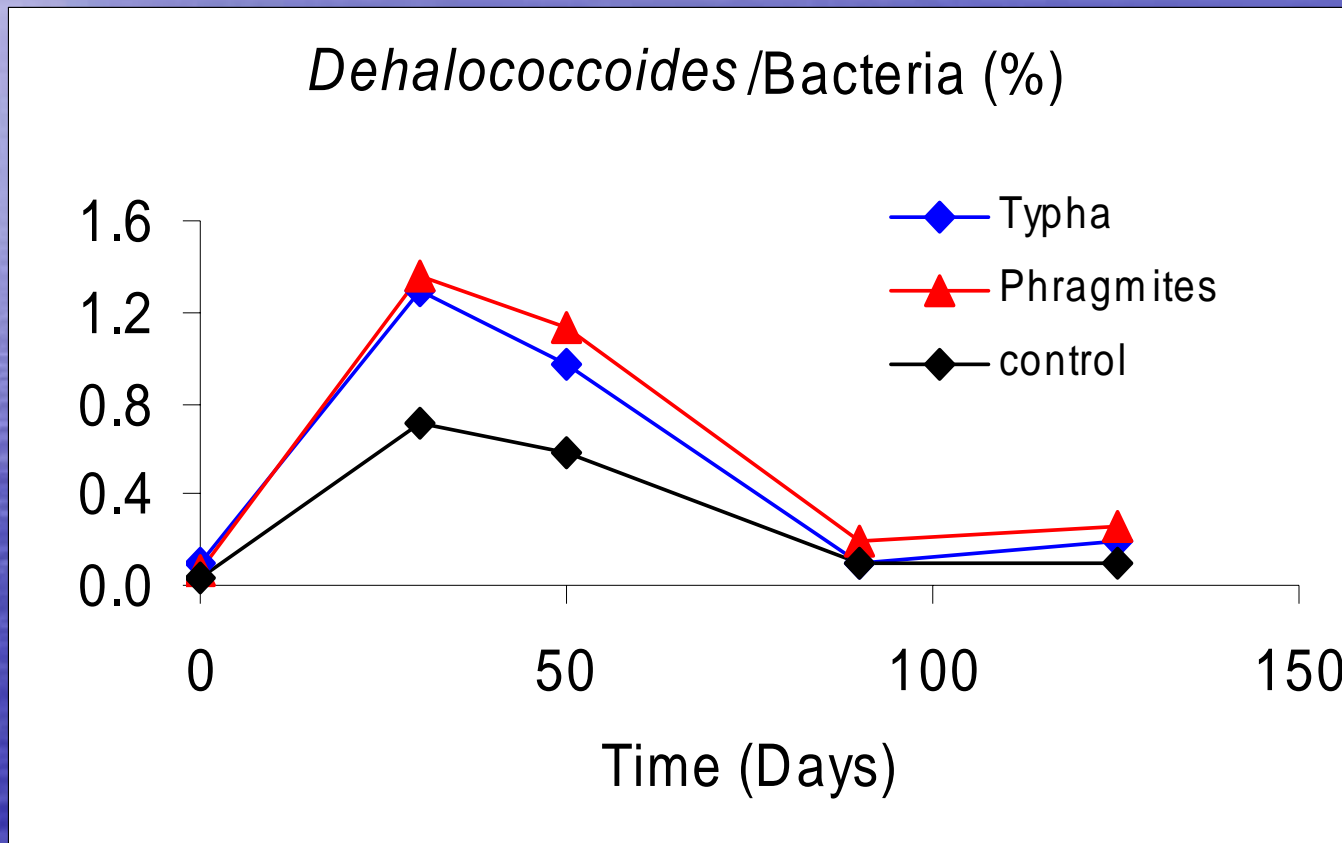
Dehalococoides in the Bulk Sediment



Eubacteria in the Bulk Sediment



Results: *Dehalococcoides*/Bacteria in sediment over time



Results: Day 125

Items	Typha		Phragmites	
	roots	sediment	roots	sediment
Eubacteria	2.16×10^9	2.35×10^8	7.78×10^8	4.14×10^8
<i>Dehalococcoides</i>	2.87×10^7	4.58×10^5	3.27×10^7	1.07×10^6
Ratio (%)	1.30	0.19	4.20	0.26

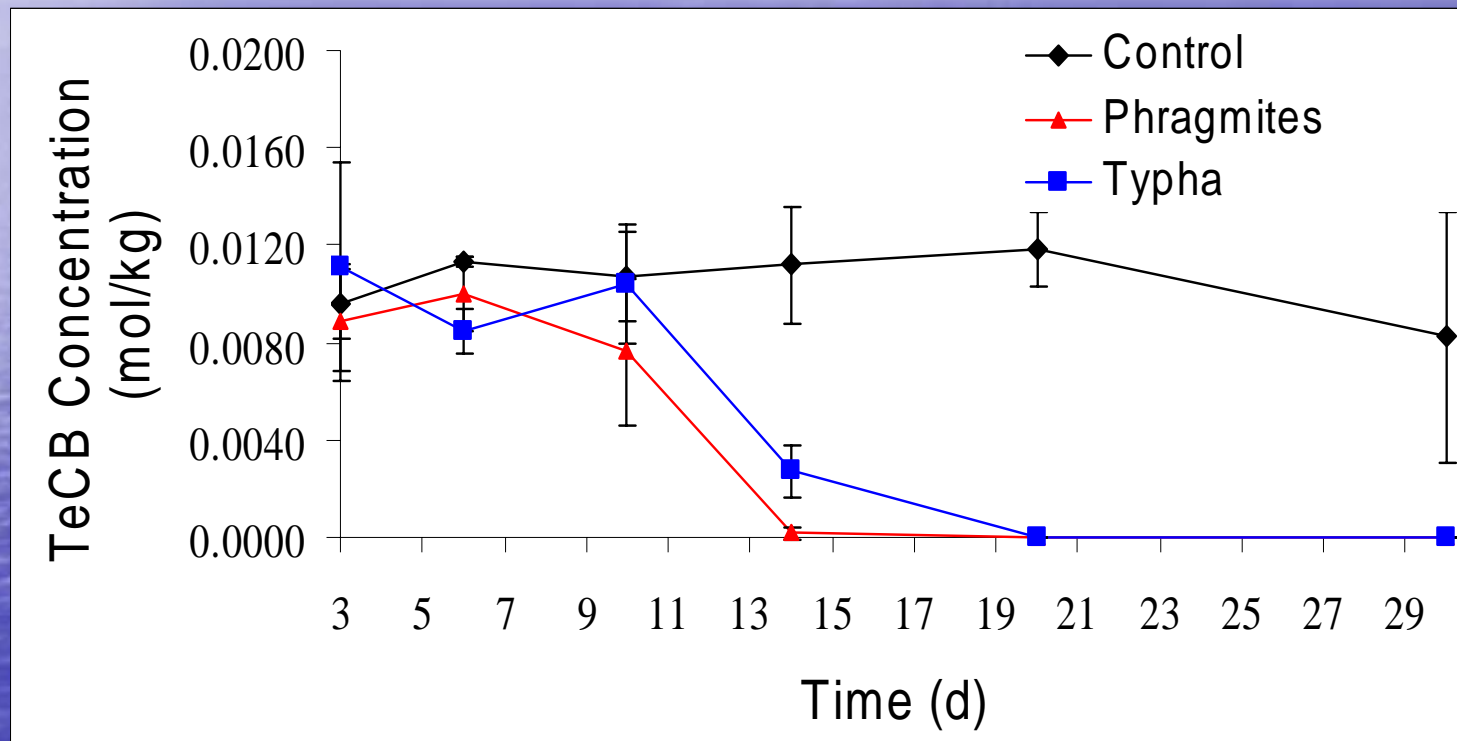
Conclusions

- Both aquatic plants facilitate reductive dechlorination of HCB
- Plants increased the bioavailability of aged contaminants
- RT-PCR is a useful technique to quantify specific microbial groups
- Plants selectively enriched certain microorganisms
- Plant roots provide locus for dehalorespiring bacteria except for exudates stimulation

Questions

Questions???

Plant-facilitated TeCB degradation



Hydrogen and Methane Production

