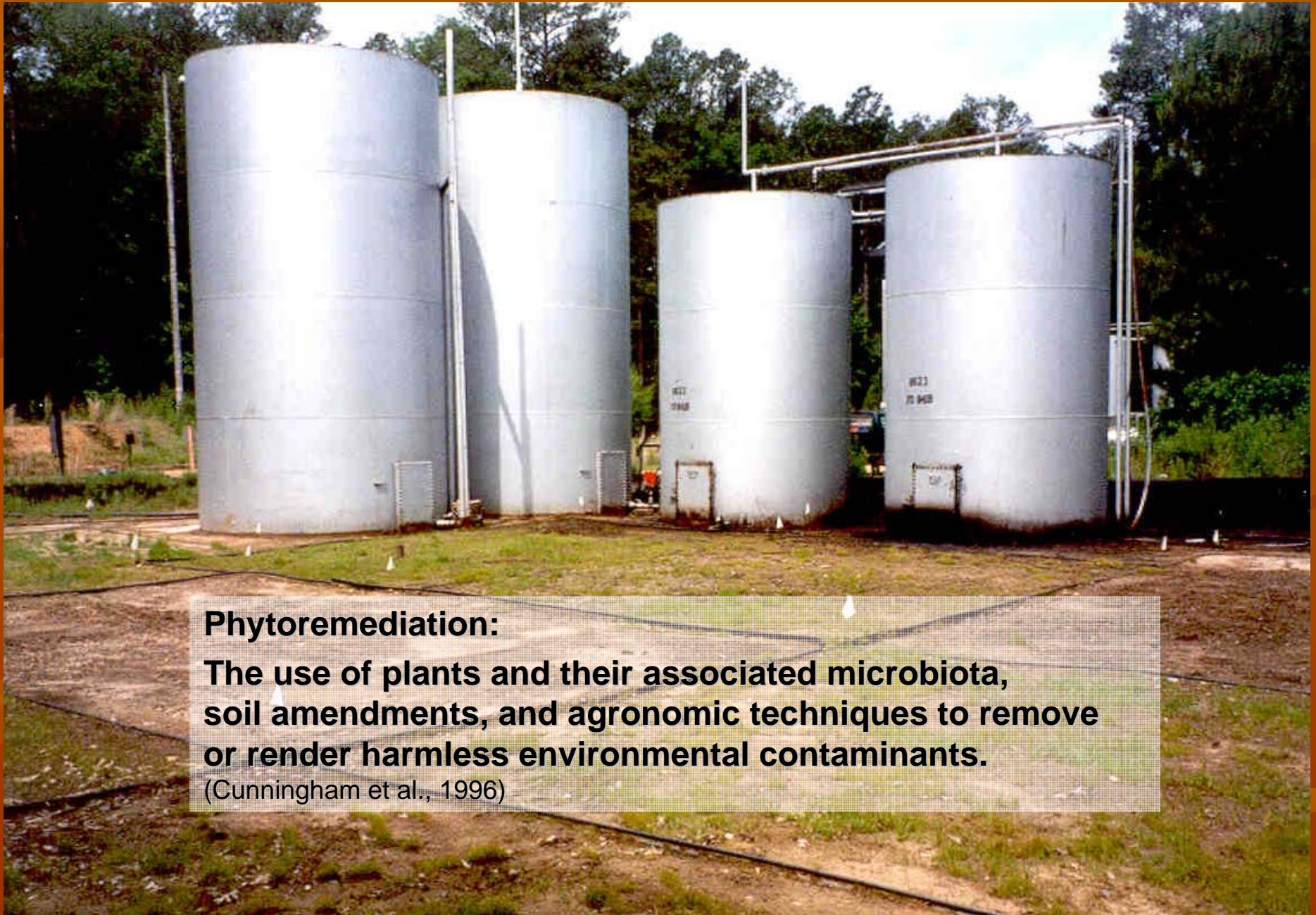


Novel Approach to Measurement of Rhizosphere Effect in Phytoremediation

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Phytoremediation:

The use of plants and their associated microbiota, soil amendments, and agronomic techniques to remove or render harmless environmental contaminants.

(Cunningham et al., 1996)

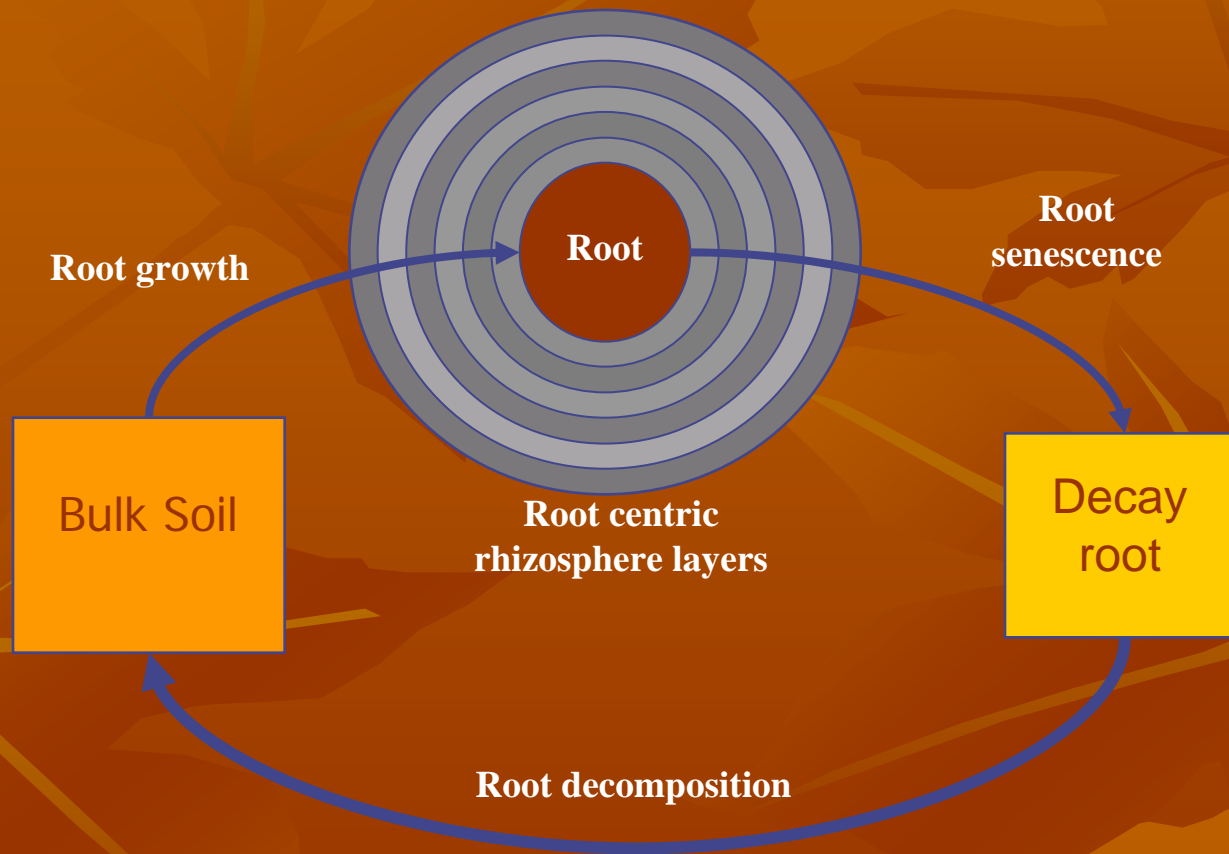
Rhizodegradation

- Carbon enriched environment
 - Exudates/lysates
- Higher microbial #s and activity
- Root 'exploration' of the soil

Rhizosphere interactions stimulate contaminant degradation by enhancing soil physical, chemical, and biological properties (adapted from Reynolds et al., 1999)

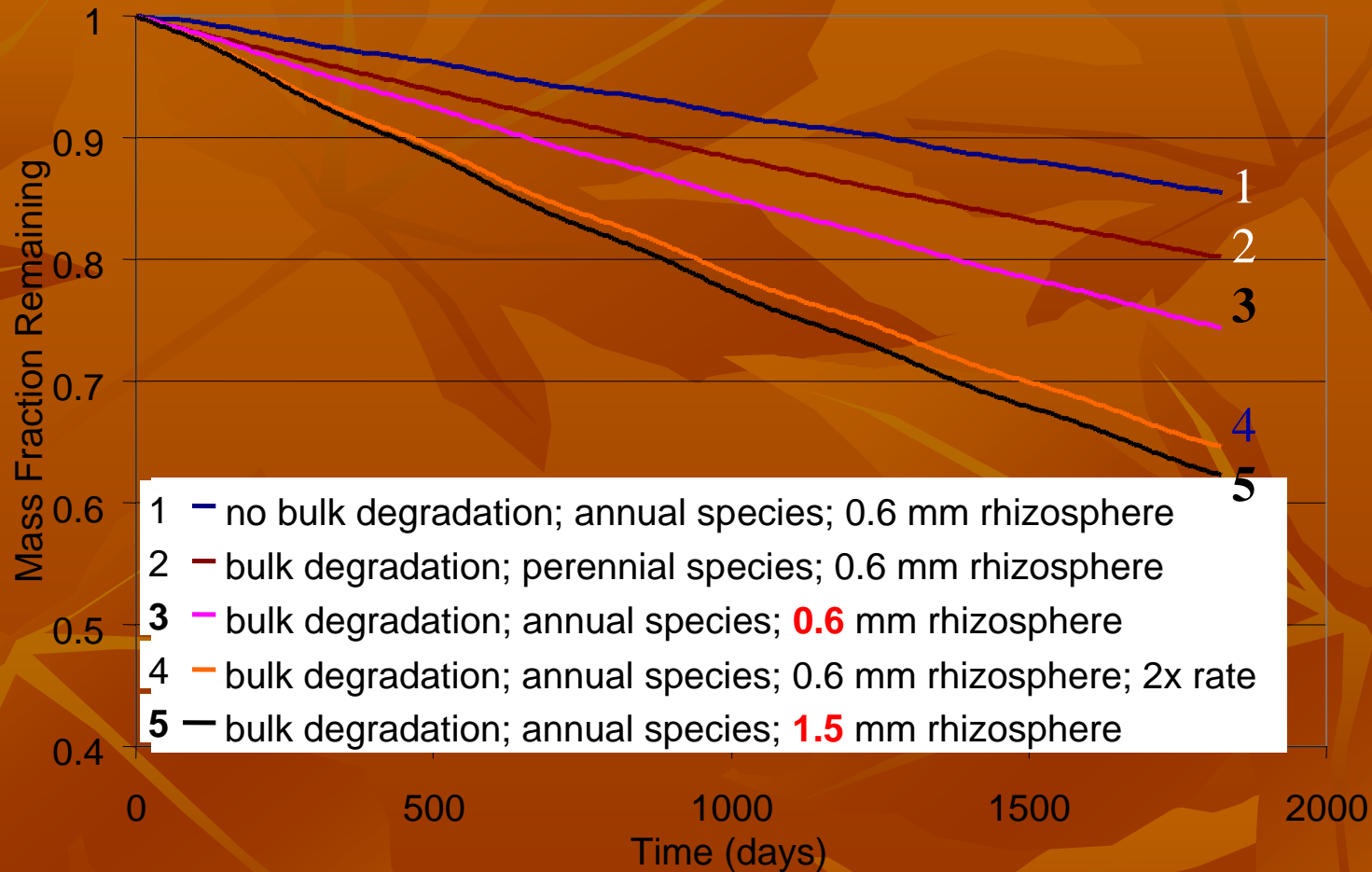


Conceptual Mathematical Model



Volume and concentration variations: 18 differential equations

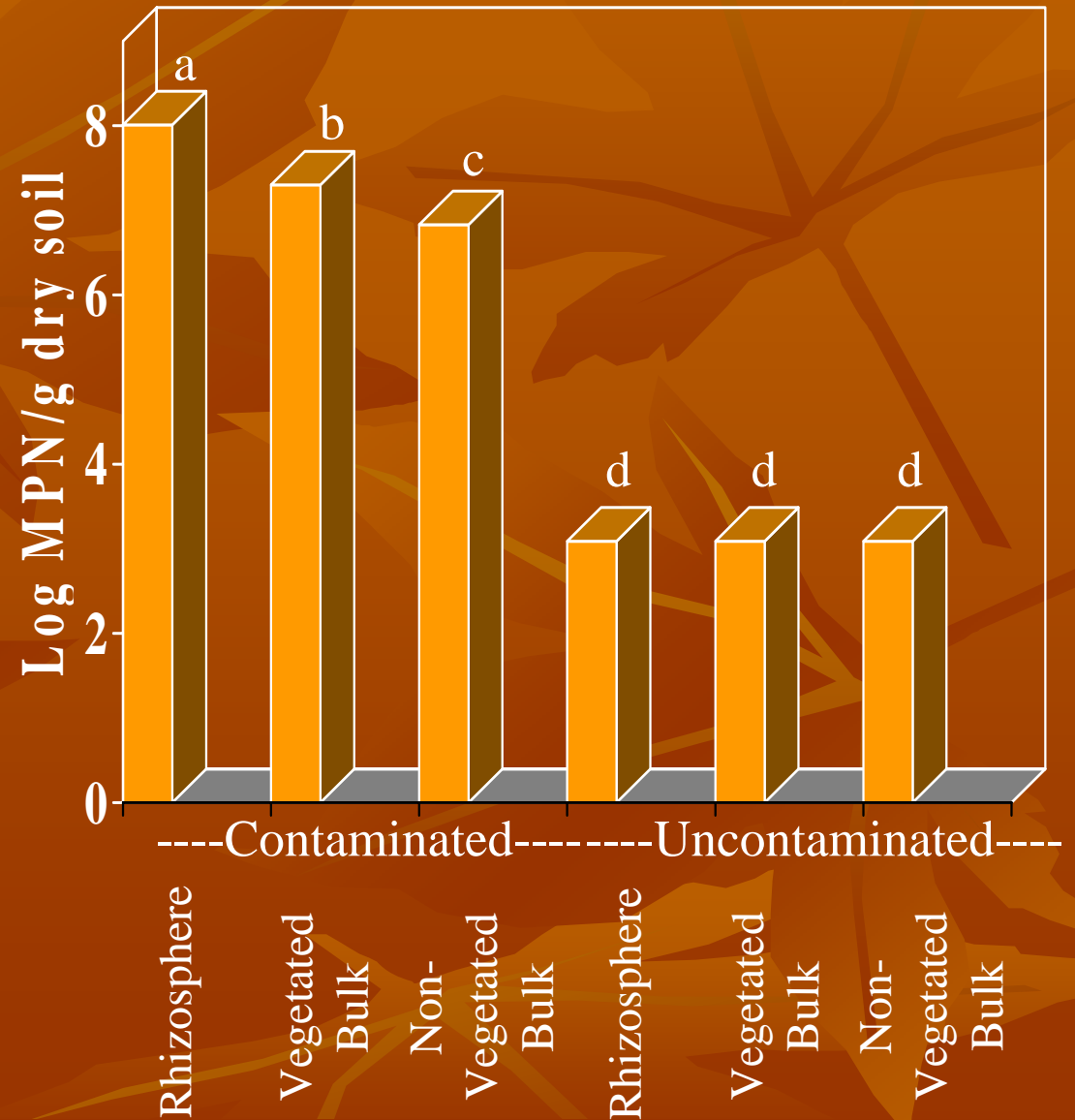
Sensitivity Analysis of Mathematical Model



- The rhizosphere degradation rate constant is kept constant at a value of 0.001 day⁻¹ (Schwab et al., 1995) with the exception of curve 4 where it was given a value of 0.002 day⁻¹. The average root volume is 0.2% of the total soil volume (White et al., 2001).

Pyrene Degradator Population 62 Days After Planting

Roxana fine sandy loam
Alfalfa, bermudagrass,
fescue,
annual ryegrass,
plant species mixture,
non-vegetated
Pyrene at 500 mg kg⁻¹
Greenhouse: 12/12 h;
25 ± 3°C
Harvested 48, 55, 62,
and 69 d



Pyrene concentrations soil 62 days after planting

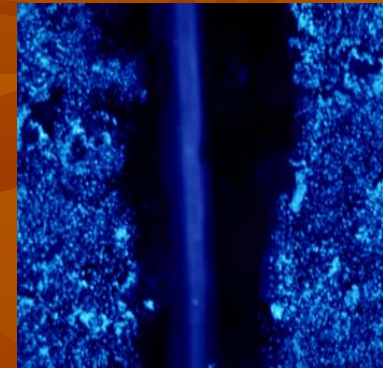
Treatment	Vegetated bulk (mg kg ⁻¹)	Rhizosphere
Alfalfa	74	19
Bermudagrass	48	6
Fescue	141	229
Ryegrass	46	88
Plant Species Mix	53	94
Non-Vegetated Bulk	99	99

Characterization of Rhizosphere Properties

- Investigated properties:
 - Microorganism population, root exudates, water, nutrients, contaminant degradation.
- Destructive methods:
 - Remove grass
 - Gently shake to remove bulk soil
 - Adhering soil defined as the rhizosphere
 - May not be completely representative of rhizosphere soil
- Non-destructive methods:
 - Dye indicator in agar plate coupled with optical densitometer (Ruiz and Arvieu, 1990): artificial environment
 - Computer tomography and nuclear magnetic resonance.
 - SR-FTIR spectromicroscopic system (Raab and Martin, 2001): expensive, scattering of IR in soil matrix.

Objectives and Hypotheses

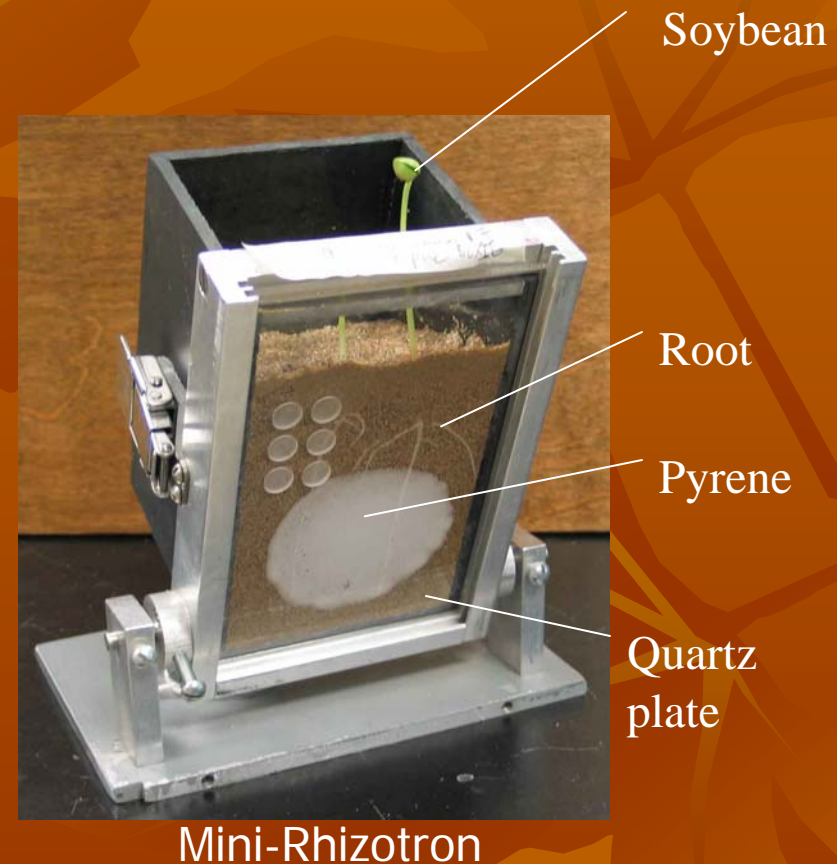
- Objectives:
 - Determine in-situ rhizosphere thickness.
 - Determine in-situ PAH degradation rate.
- Hypotheses:
 - Contaminant depletion in soil zone adjacent to the root surface will be visible.
 - Differences in luminescent intensity of PAHs under UV light will be observed between rhizosphere and bulk soil and over time.



Anticipated depletion of phenanthrene in the rhizosphere

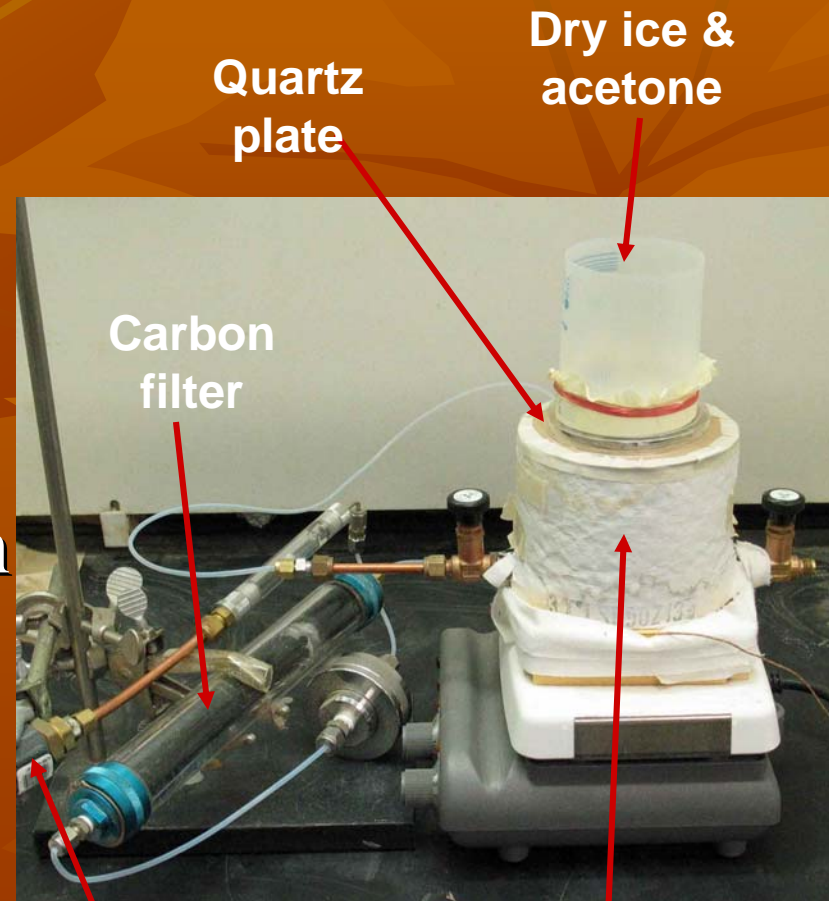
Materials and Methods

- Deposit a thin PAH film onto a quartz plate.
- Assemble a inclined rhizotron with the quartz plate on one side.
- Grow plants under controlled environment in a BOD chamber.
- Photograph PAH film
 - Digitize and perform luminescent intensity analysis



Materials and Methods (cont.)

- PAH film preparation
 - Physical vapor deposition
 - $P = 1.9$ torr.
 - Uniform fine structure film.
- Uniform heat distribution
 - Aluminum chamber.
 - Thick bottom plate and insulated wall (150°C).
- Cold substrate (quartz)
 - Dry ice and acetone (-78°C).
 - Minimizes surface diffusion.



To vacuum
pump

Insulated
Chamber

PAH Deposition Chamber

Materials and Methods (cont.)

- Image Acquisition
 - Camera: Nikon 8008s w/ Nikkor 60mm (macro)
 - UV lamp: 100W; fixed relative position
 - Bellows: Nikon PB-6E (22X magnification)
 - XY positioning platform
 - Scanner: Minolta Dimage Scan Elite 5400
 - 4000 dpi (20 Mpixels)

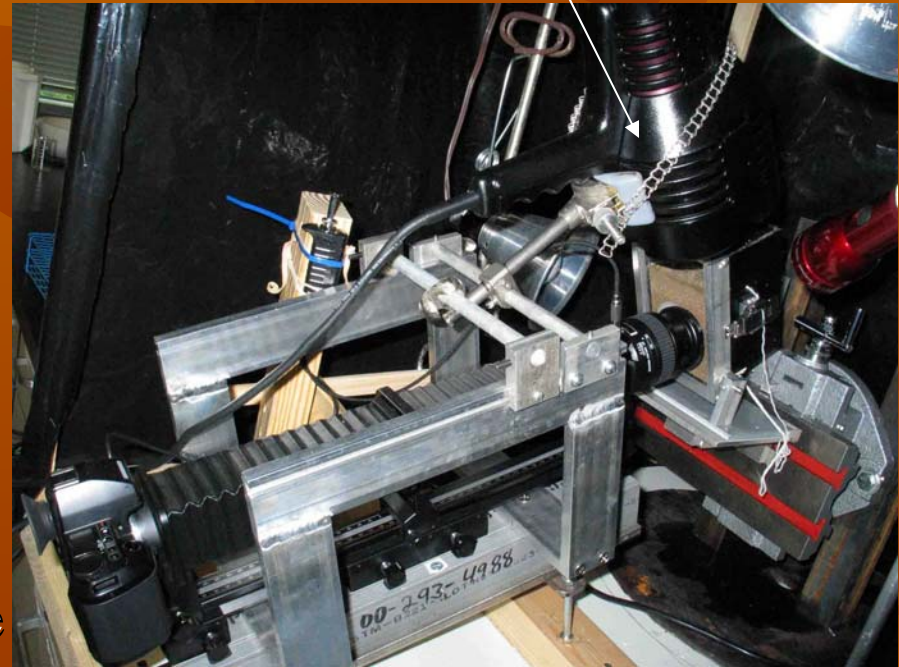
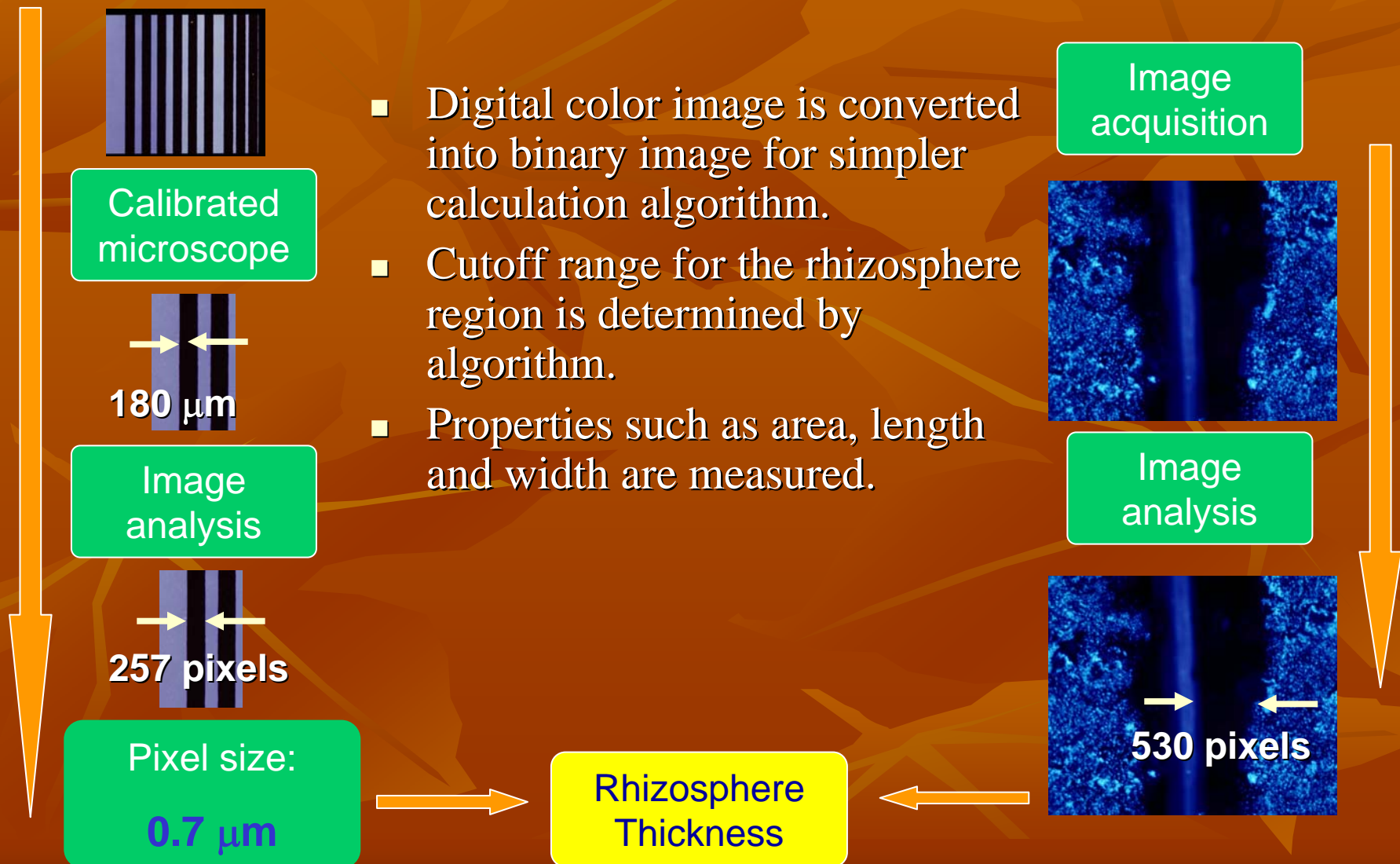


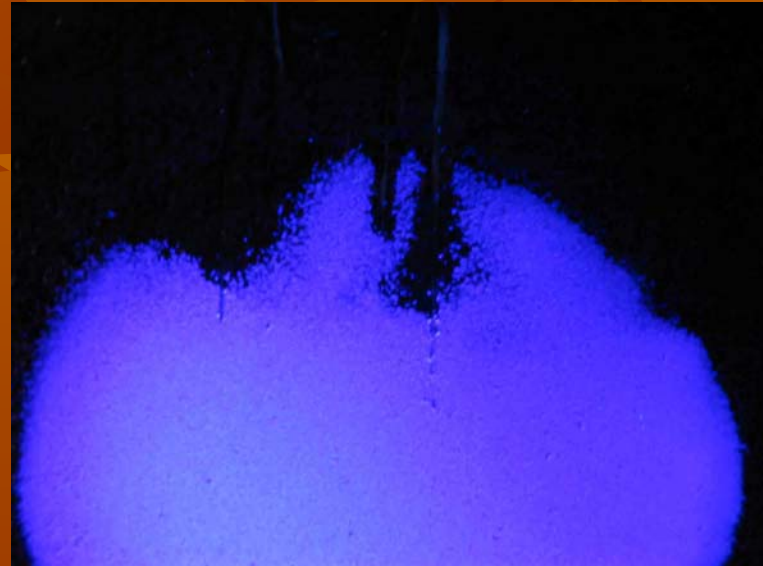
Image Calibration and Rhizosphere Thickness Calculation



Soybean on Phenanthrene (3 weeks)

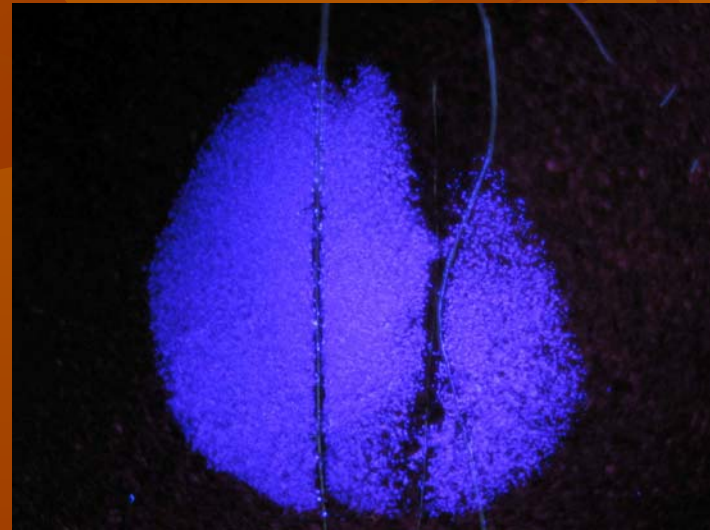
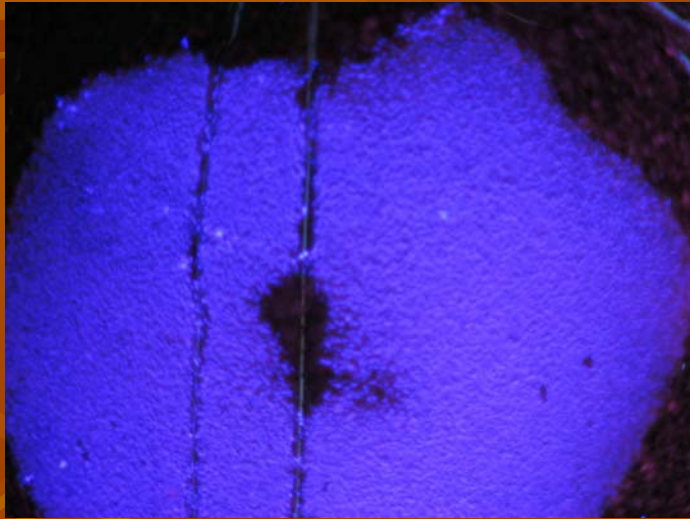


Visible light illumination

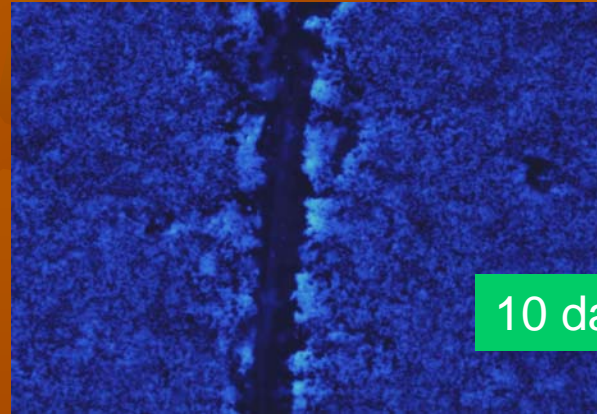


Ultraviolet light illumination

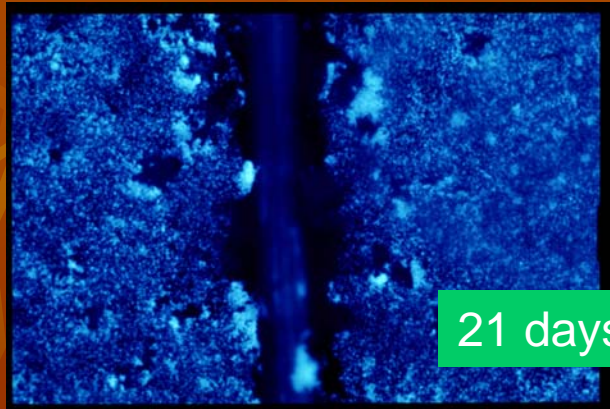
Soybean on Phenanthrene (4 weeks)



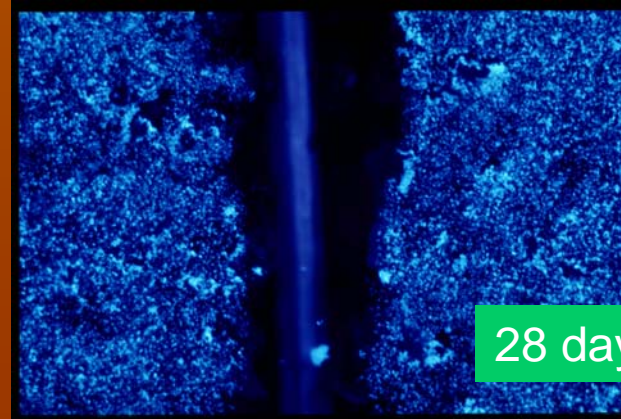
Close-up Visualization of Rhizosphere: Soybean on Phenanthrene



10 days



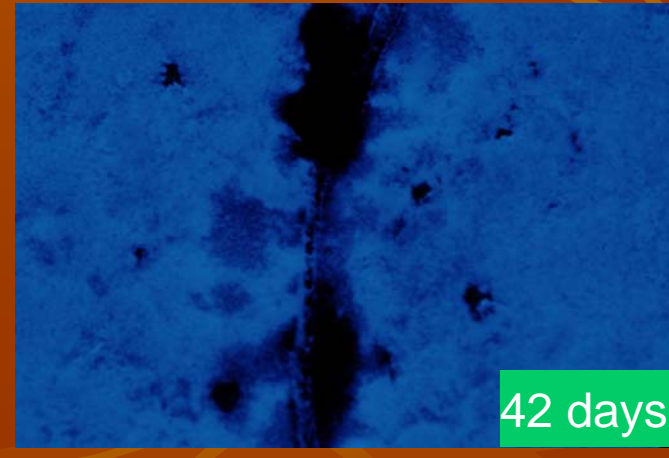
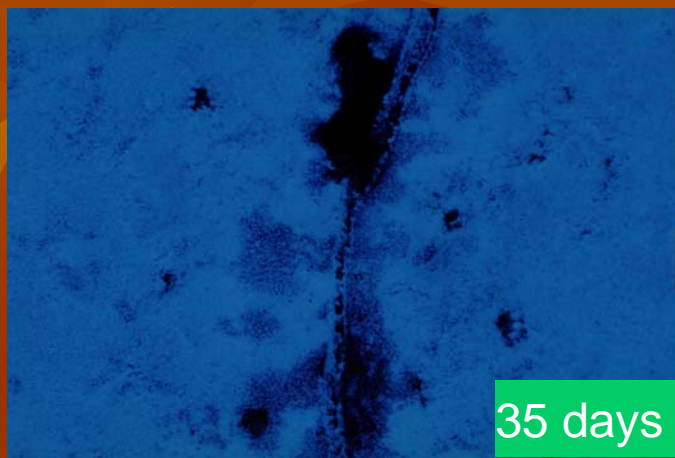
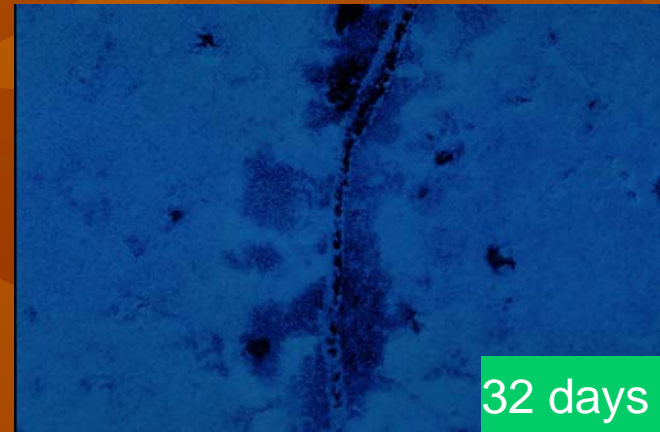
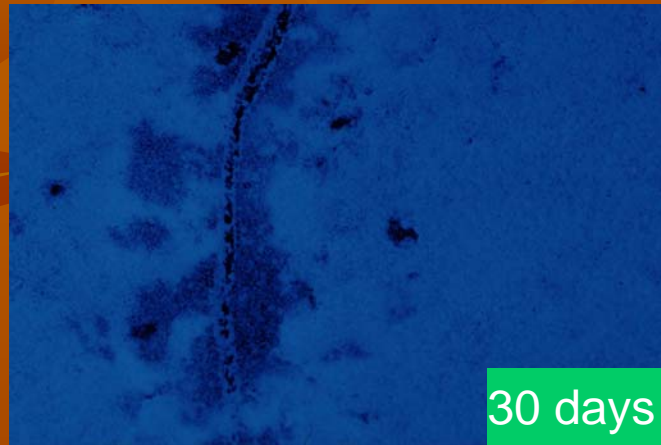
21 days



28 days

2.5 mm

Development of Bermuda Rhizosphere on Pyrene



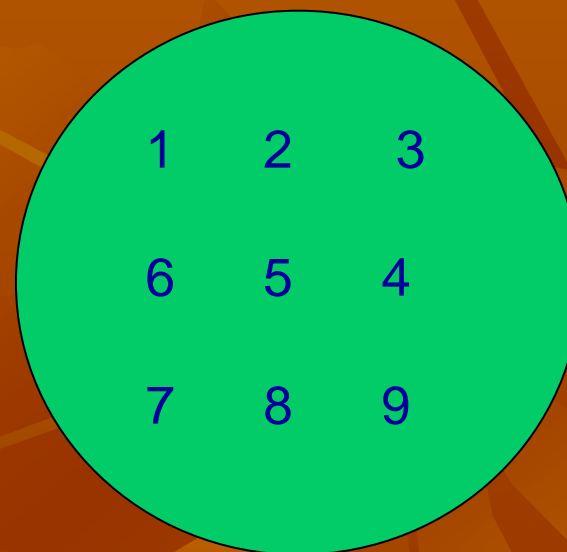
Root and Rhizosphere Dimension

	Rhizosphere Thickness (μm)	Root diameter (μm)
Soybean + phenanthrene	370	400
Bermuda + pyrene	280	140

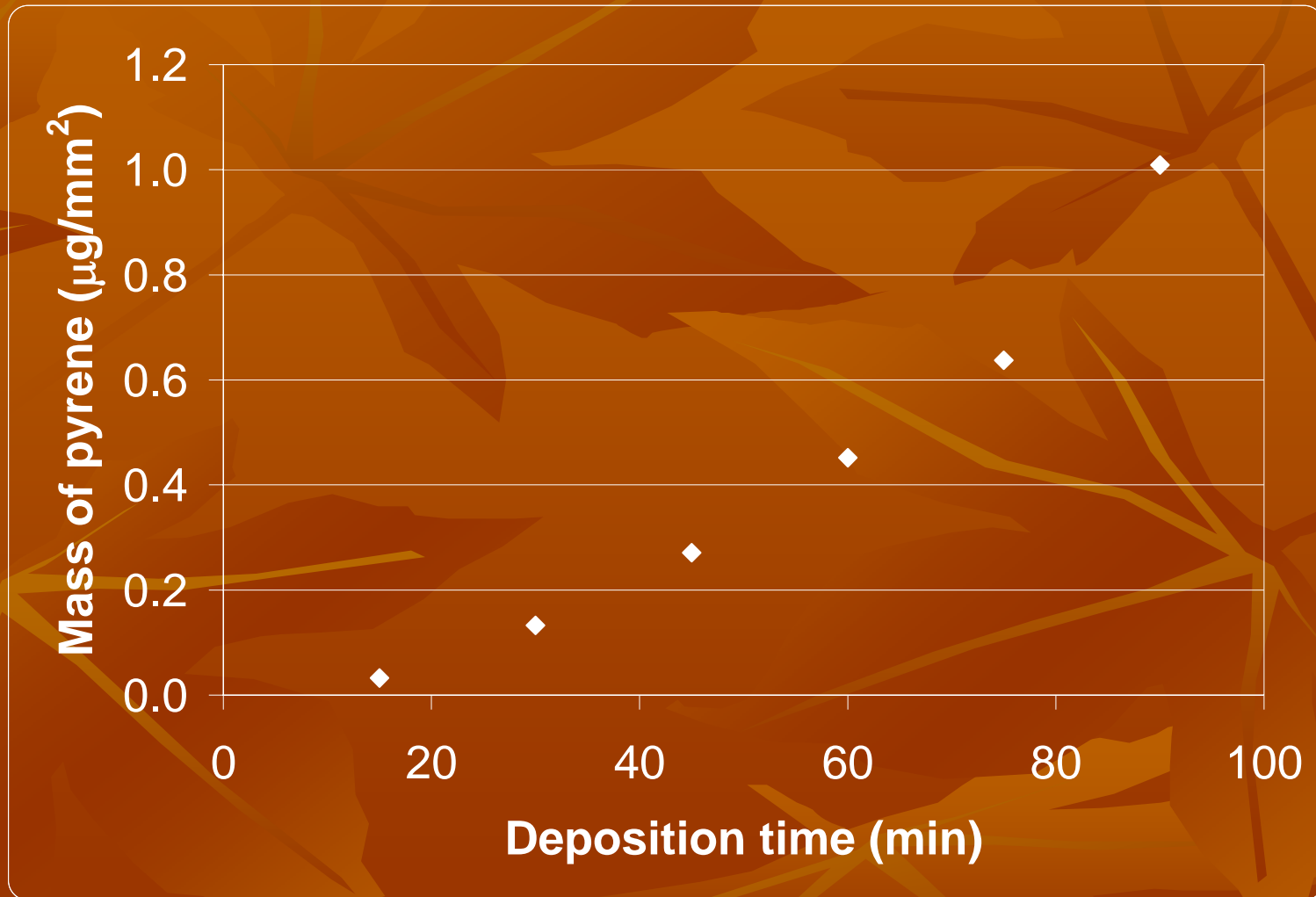
Rhizosphere thickness defined as the zone of clearing

Quantitative Image Analysis: Pyrene Film Uniformity and Luminescent Intensity Calibration

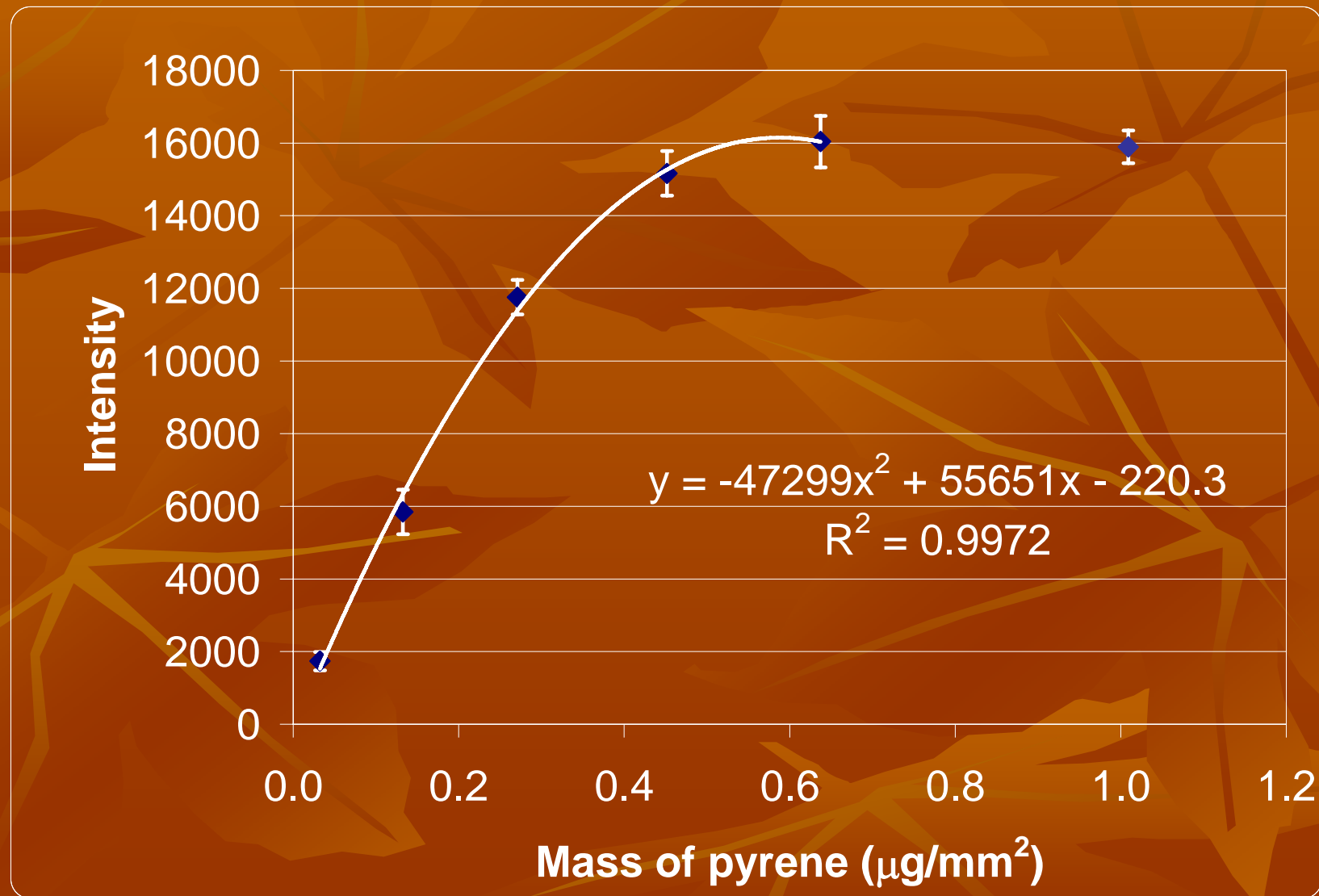
- Deposit pyrene thin films with different deposition time.
- Take UV illuminated photographs at 9 positions on the deposited pyrene thin film.
- Calculate of the image's intensity with Matlab program.
- Calculate of mean and standard deviation of the intensity.
- Dissolve the pyrene film with acetone and analyze pyrene concentration or mass with HPLC



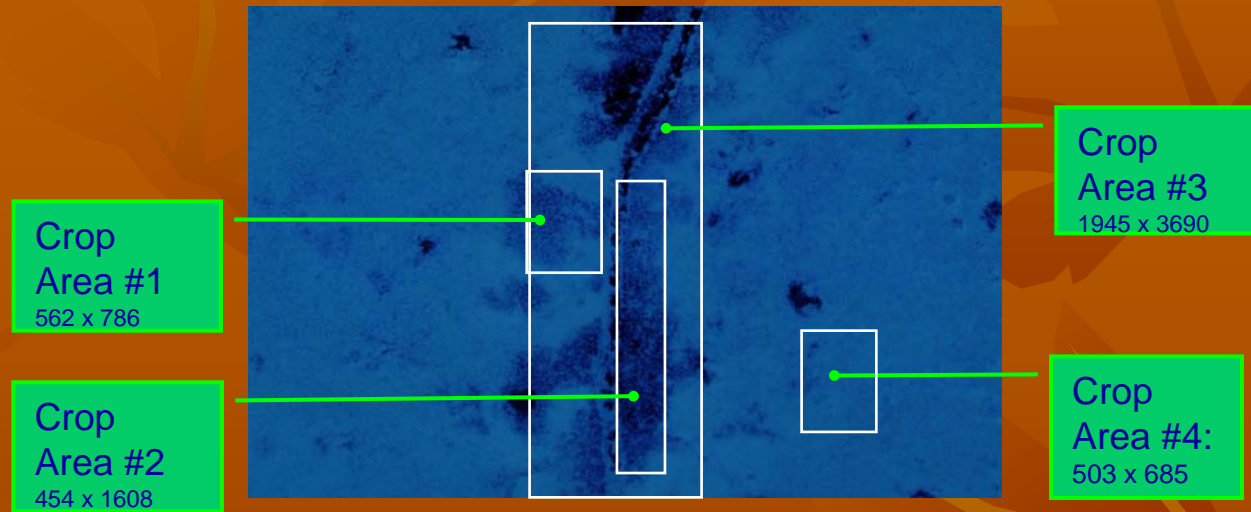
Mass of Pyrene and Deposition Time Correlation



Luminescent Intensity vs. Pyrene Mass

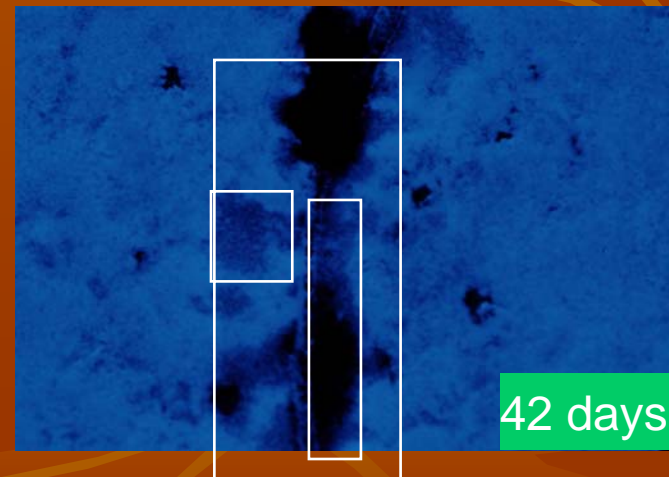
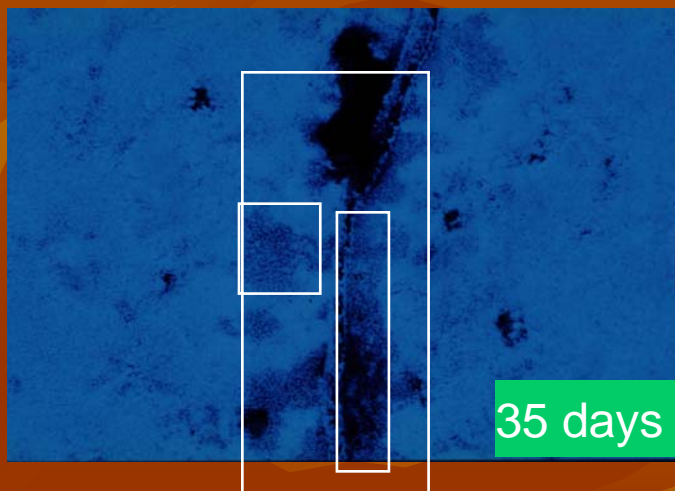
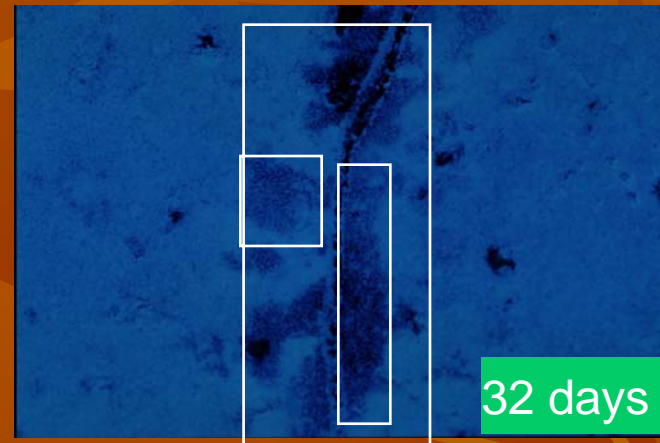
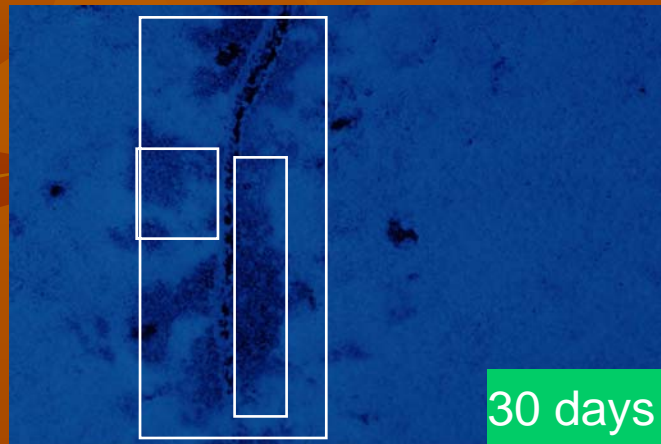


Rhizosphere Contaminant Degradation Rate

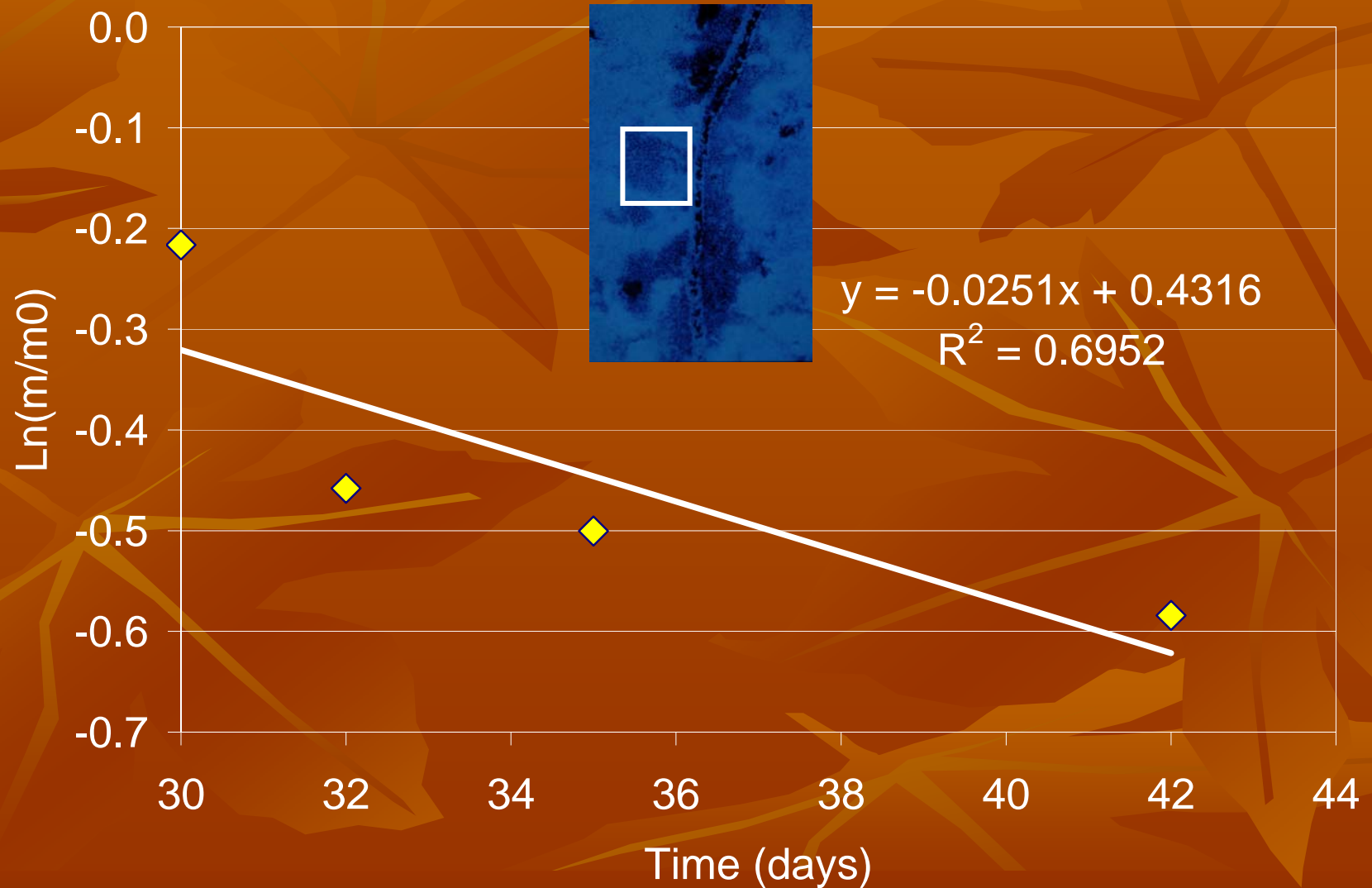


- Photograph rhizosphere over time.
- Crop the image at different positions.
- Calculate the crop image luminescent intensity.
- Calculate mass of pyrene remaining.
- Calculate degradation rates

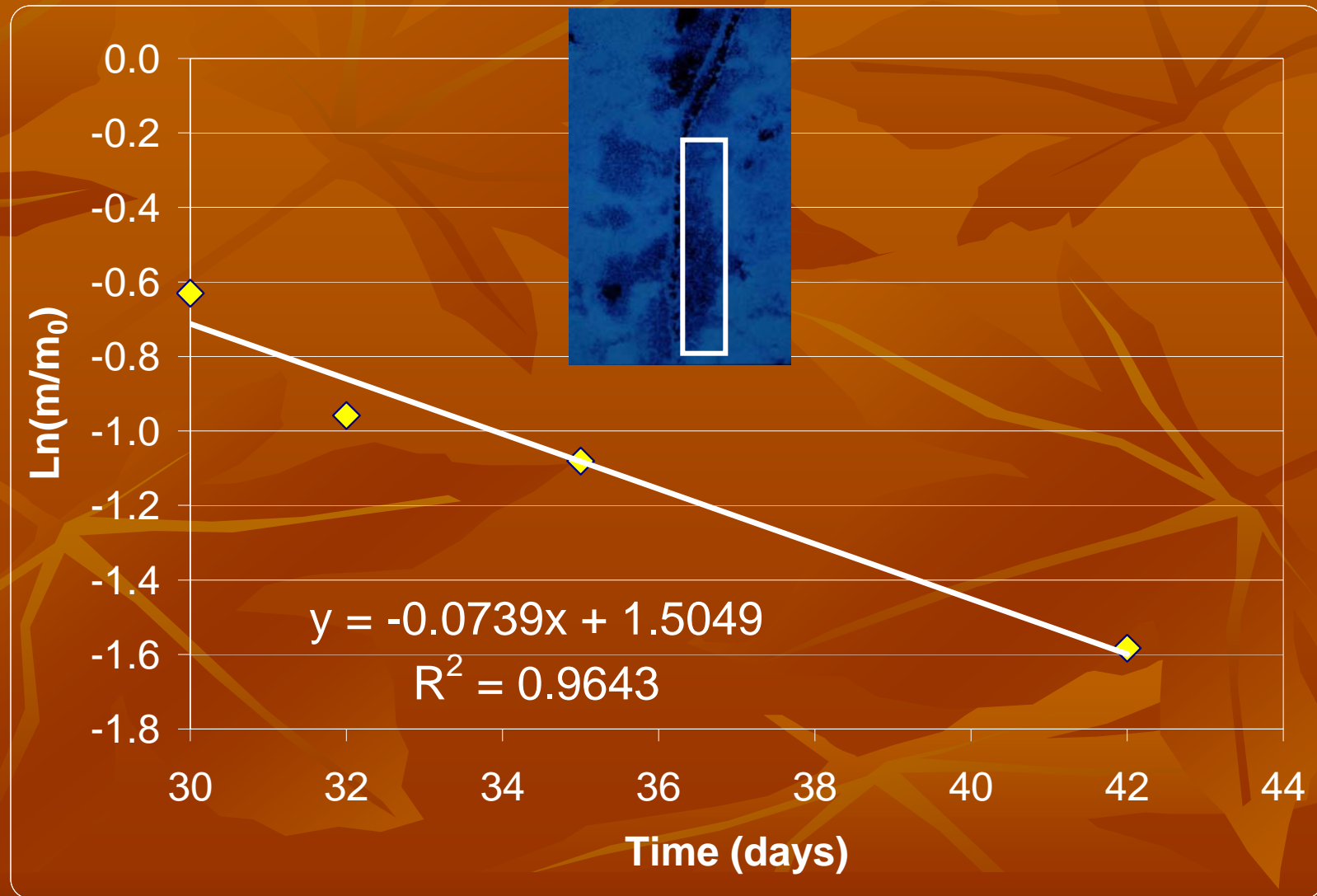
Bermuda Rhizodegradation Rate Constant



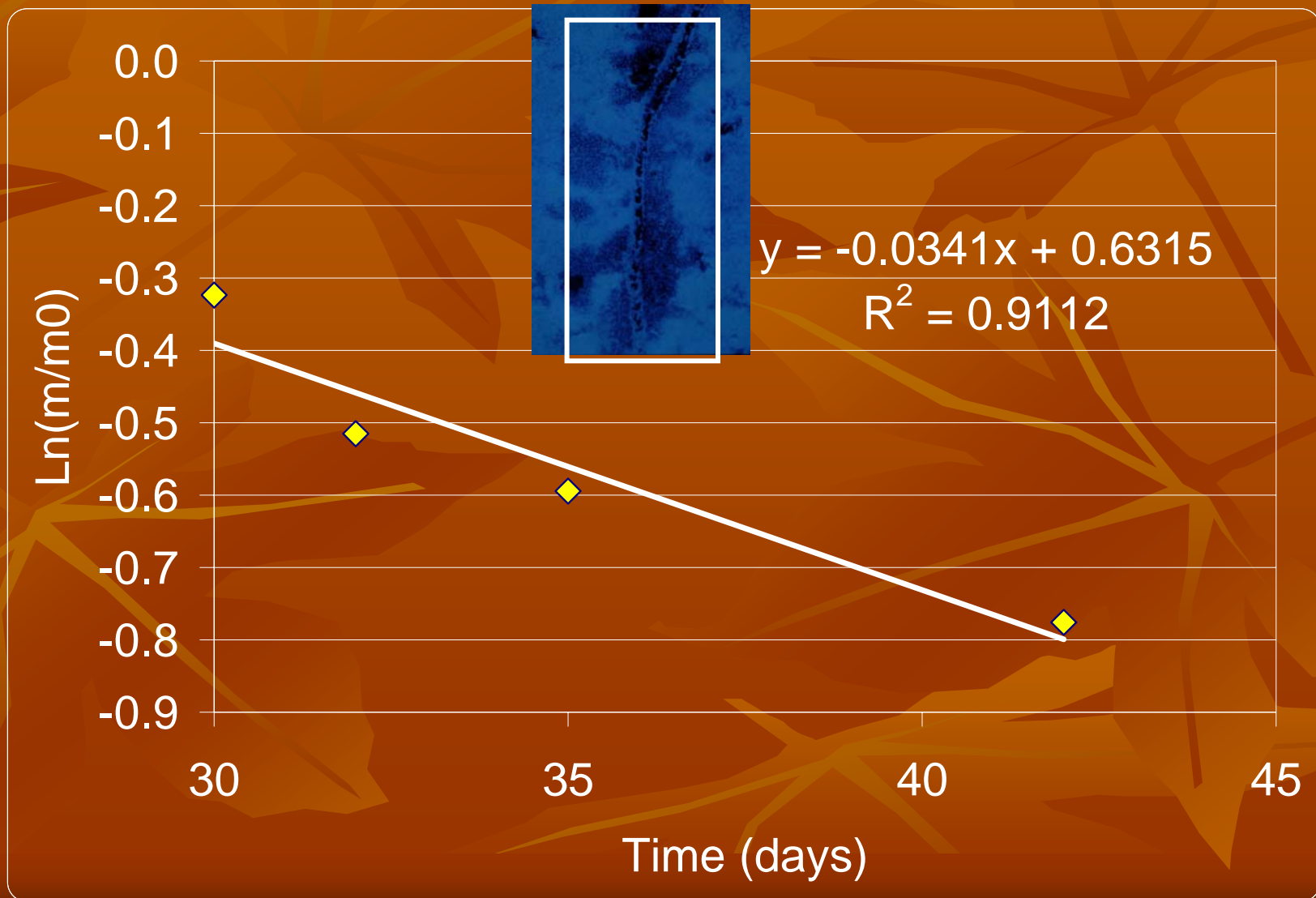
Degradation Rate: Zone 1



Degradation Rate: Zone 2



Degradation Rate: Zone 3 - Entire Rhizosphere



Conclusions

- Non-destructive, non-invasive quantification of rhizosphere thickness and in-situ PAH degradation rate are possible.
- Rhizosphere thickness and contaminant degradation:
 - Expand over time.
 - Vary along the root.
 - Depends on plant type, plant growth condition, soil structure and other factors.
- Understanding contaminant dynamics in rhizosphere is needed for modeling efforts.

Acknowledgement

- This project is funded by the EPA through the Integrated Petroleum Environmental Consortium.

Thank you