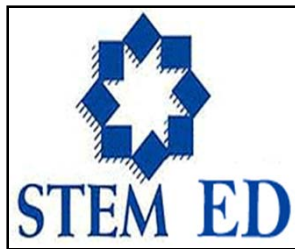




# Leaf Leaching Experiment

Monday PM



David A. Reckhow  
University of Massachusetts

# Introduction

- Water treatment engineers and public health officials need to pay careful attention to the presence of dissolved organic matter in water supplies selected for human consumption (i.e., raw drinking waters).
  - For many reasons related to human health and cost, it is necessary to remove a substantial amount of these organic compounds before the water is ready for human use. The cost of doing this is highly variable and dependent on the particulars of the ecosystem from which the raw water originates.
  - In the Northeast US, a large fraction of aquatic organic matter found in lakes and rivers comes from the forest floor. We're all familiar with organic-rich soils with upper soil horizons containing roots, branches, logs and leaves in various stages of decay.

# Intro (cont.)

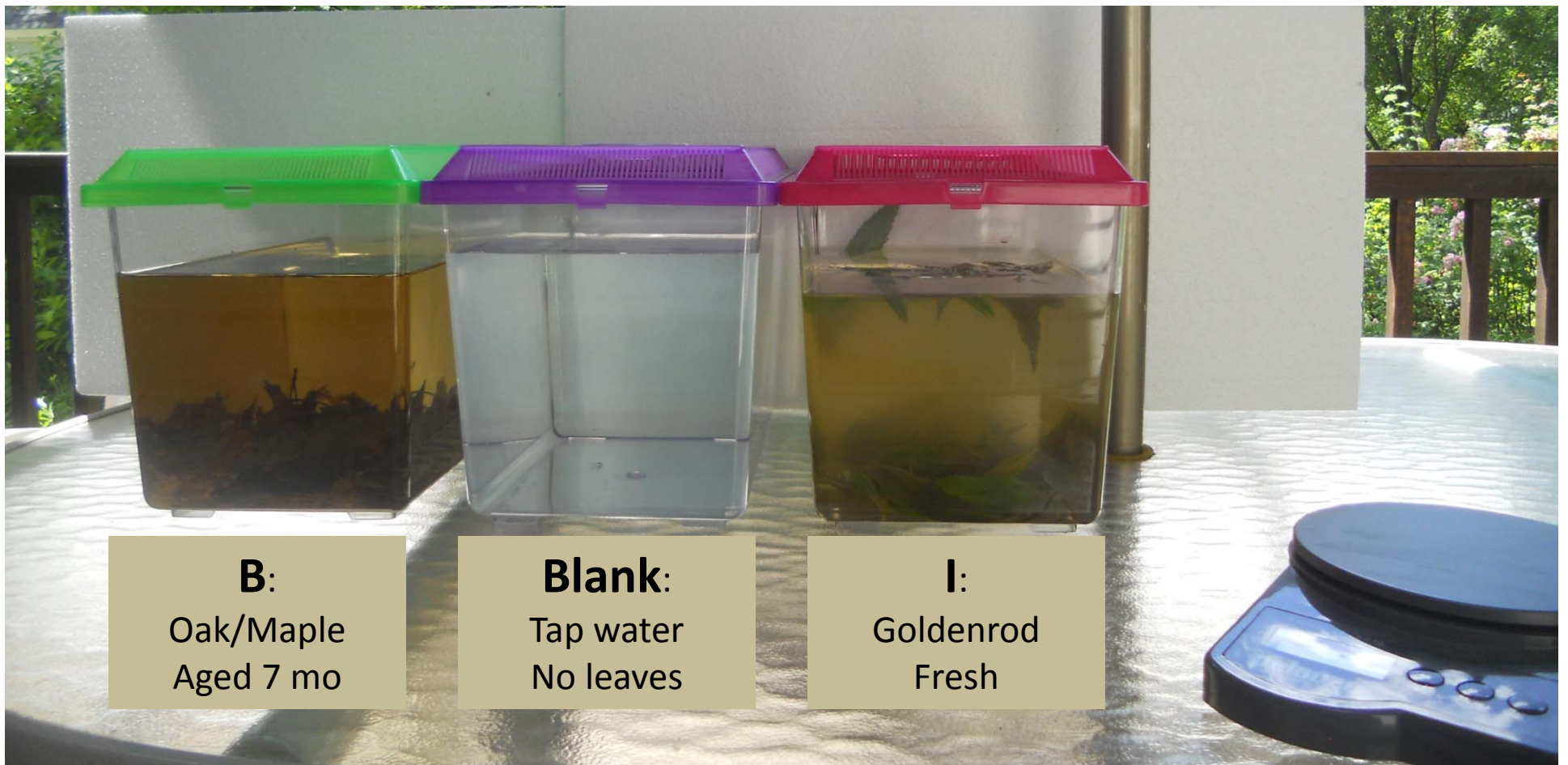
- Thinking about the fate of plant biomass, many questions come to mind.
  - How much of this plant biomass degrades to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in place (i.e., the process of mineralization), how much becomes incorporated in the soil below and how much becomes dissolved in pockets of water and is carried away to groundwater and surface water bodies (i.e., solubilization)?
  - How quickly does this happen and do leaves age and degrade at the same rate for all species under all conditions?
  - What are the steps in degradation and solubilization?
  - Does decomposition/degradation and solubilization occur at a constant rate, or an increasing or decreasing rate with time? Is there a lag phase?

# Materials

- Containers for leaching & imaging
  - Plastic Culture Flasks (275 mL),
    - Corning #430720; \$3.70 each from Fisher Scientific
  - Plastic Aquarium
    - Kritter Keeper (Large), Lee's Aquarium & Pet Products
    - \$14 from local pet store
- Scale (reading in grams)
  - I used a postal scale, but top loading balance is better
- Plant Biomass
  - Your backyard
- Tap Water
- Camera, computer & ADI software

# 10 L aquaria

- About 7 g of leaves





Spatial Analysis

Enhance Colors

Mask Colors

Check Color Quality



About

Spatial tools measure the color and size of features in digital images.

Select Version of Image to View and Analyze

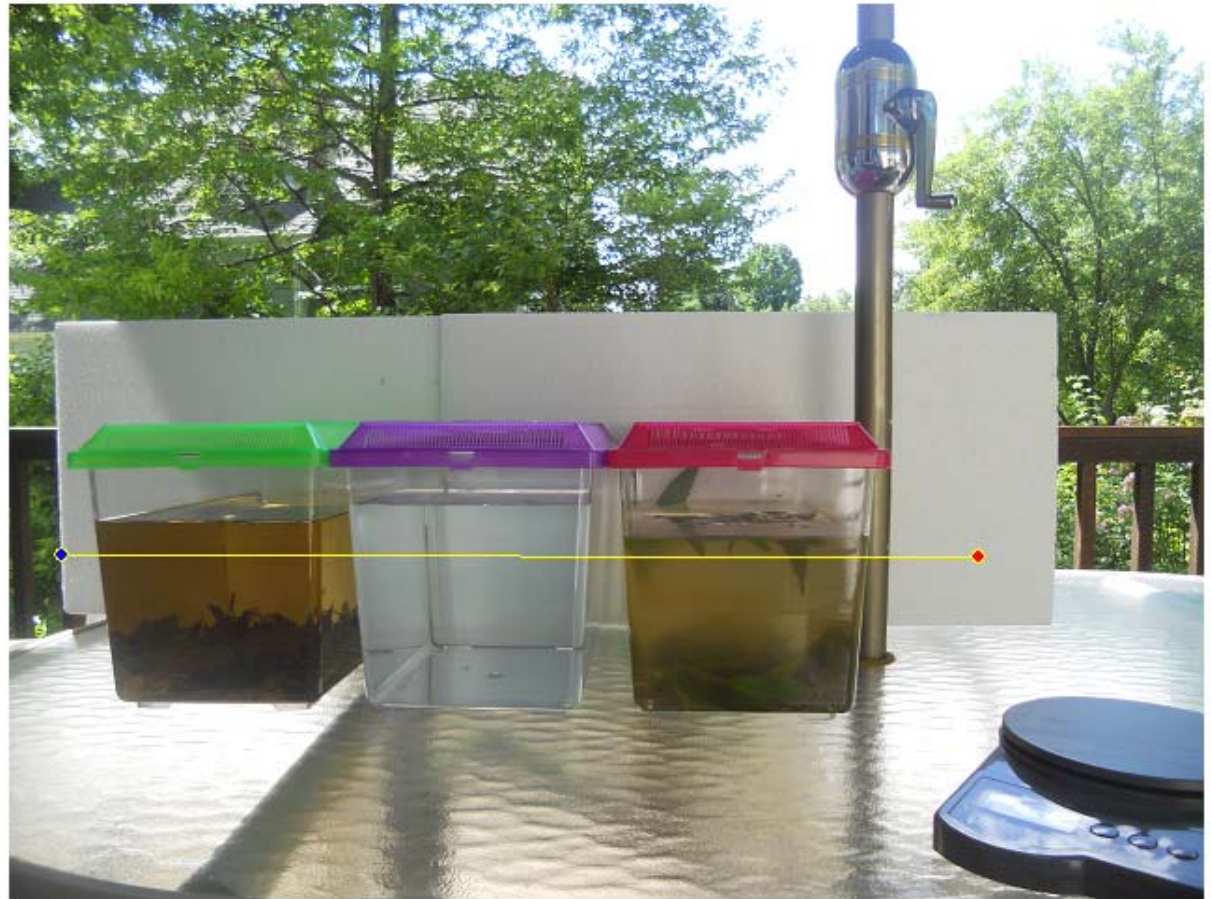
Original  Enhanced  Masked

Line Tool

	Pixel Position		Adjust
	X	Y	
Start Point	28	295	
Stop Point	519	296	
Number of Pixels	492		

Color	Intensity [%]
Average Red	48.11
Average Green	44.77
Average Blue	34.33
Average Color	42.

Intensities of colors range from 0%, meaning none of the color is present, to 100%, when maximum color is present.

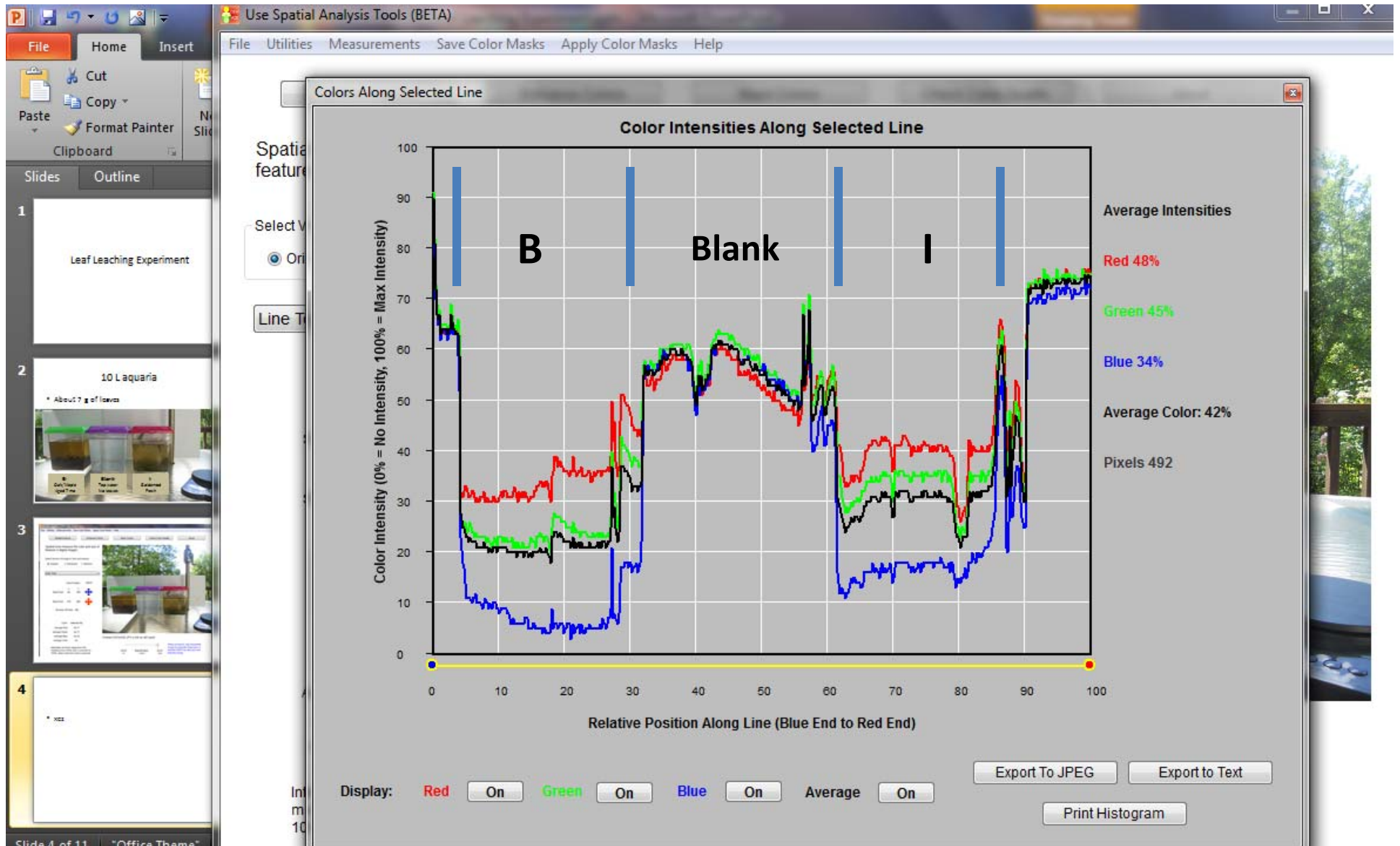


Trimmed DSCN4382.JPG is 640 by 480 pixels

Zoom In      Magnification: 1.00 x      Zoom Out

When zoomed in, pan around the image by using the arrow keys or hold the SHIFT key and click and drag the image.

# Line Tool: Aquaria



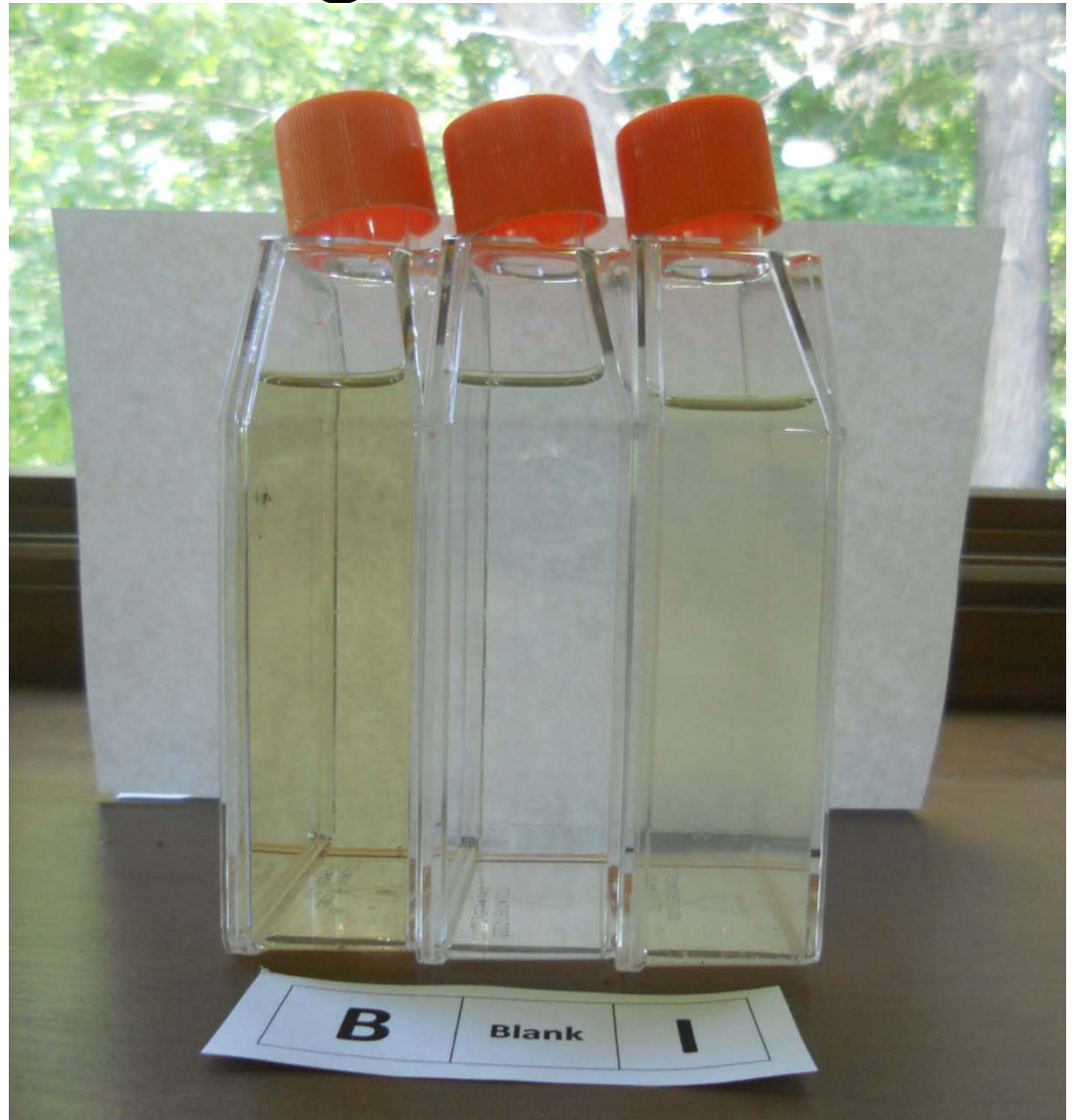
# Aquaria vs Bottles

- Different Pathlengths
  - 9 cm for bottles
  - 32 cm for aquaria (better for low level leaching)
- Aquaria still have plant biomass
  - Bottles allow separation, or decantation of leaching mixture
- Aquaria are good for producing large volumes of leachate (~10L) for later testing
  - Bottles (Culture flasks) hold ~75 mL

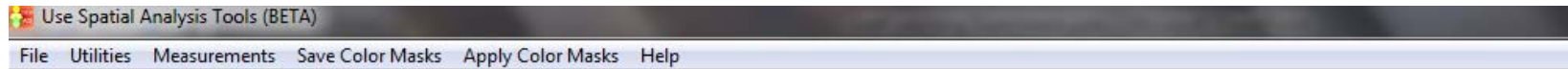


# Photographic Image: Bottles

- Blank in middle
- Experimental on either side
  - 7g/tank
    - probably too low
- White background
- ID labels below



# Line Tool



- line

Spatial Analysis Tools (BETA)  
Enhance Colors  
Mask Colors  
Check Color Quality  
About

Spatial Analysis  
Enhance Colors  
Mask Colors  
Check Color Quality  
About

Go to the Color Enhancement Window

Spatial tools measure the color and size of features in digital images.

Select Version of Image to View and Analyze

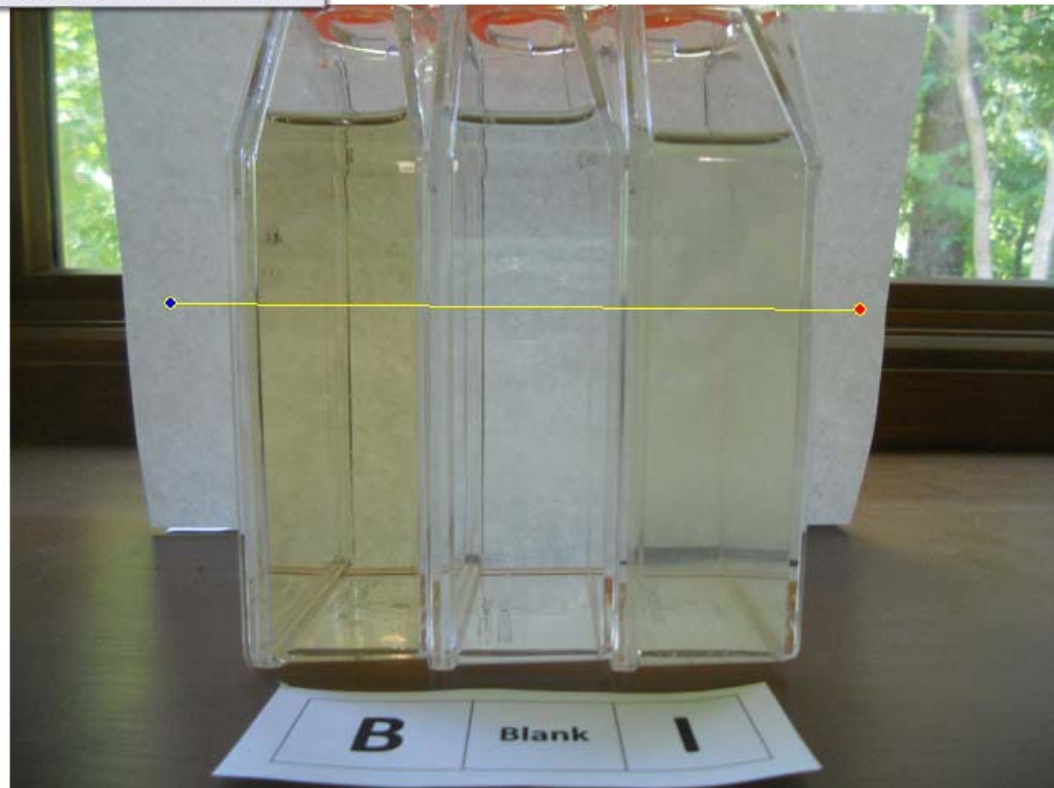
Original    Enhanced    Masked

Line Tool

	Pixel Position		Adjust
	X	Y	
Start Point	98	182	
Stop Point	519	186	
Number of Pixels	422		

	Color	Intensity [%]
Average Red		56.23
Average Green		58.28
Average Blue		52.44
Average Color		55.

Intensities of colors range from 0%, meaning none of the color is present, to 100%, when maximum color is present.

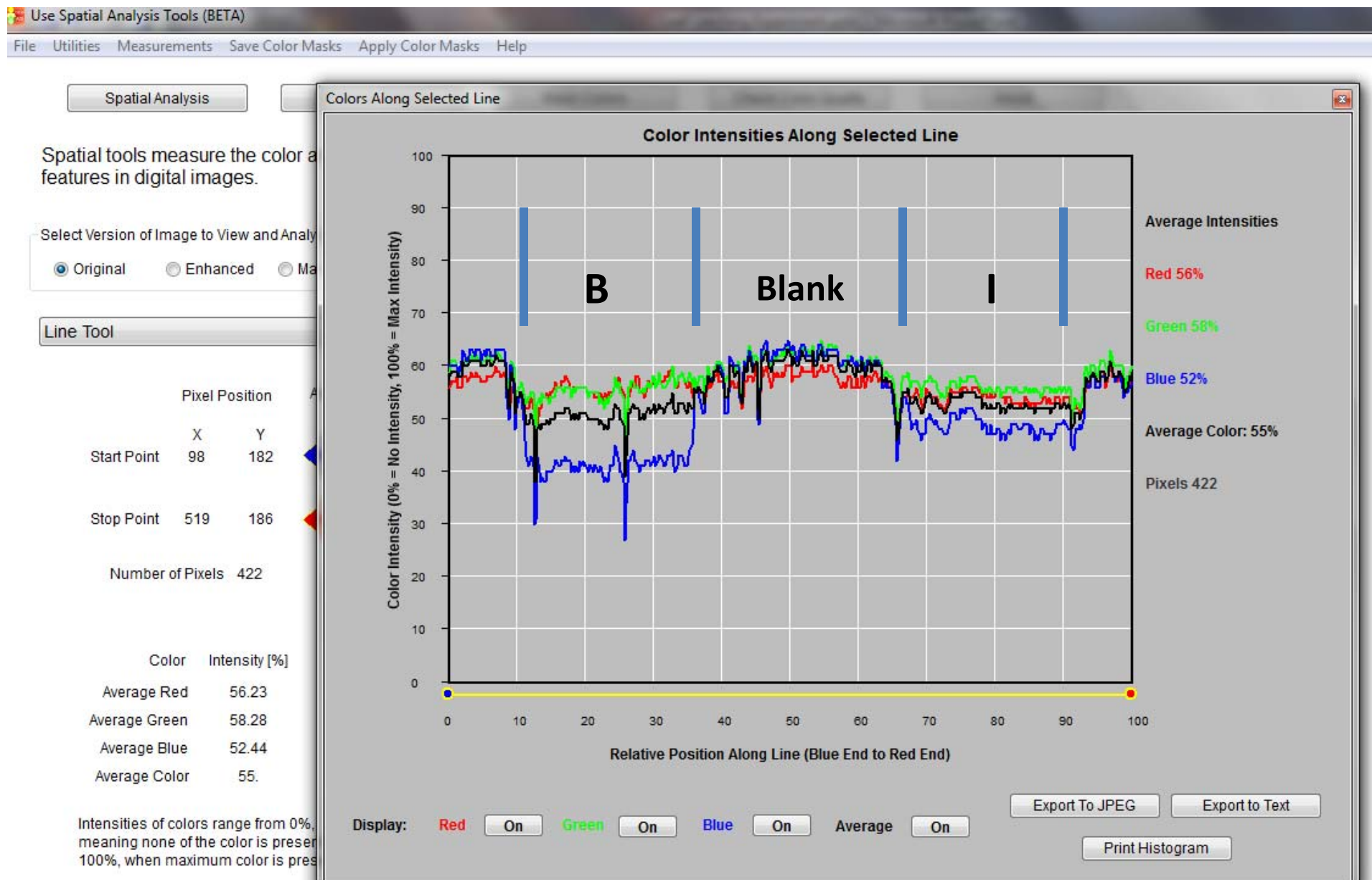


Trimmed DSCN4373.JPG is 640 by 480 pixels

Zoom In   Magnification: 1.00 x   Zoom Out

When zoomed in, pan around the image by using the arrow keys or hold the SHIFT key and click and drag the image.

# Line Tool: Color along line





# Rectangle Tool

Use Spatial Analysis Tools (BETA)  
File Utilities Measurements Save Color Masks Apply Color Masks Help



Spatial Analysis Enhance Colors Mask Colors Check Color Quality About

Spatial tools measure the color and size of features in digital images.

Select Version of Image to View and Analyze

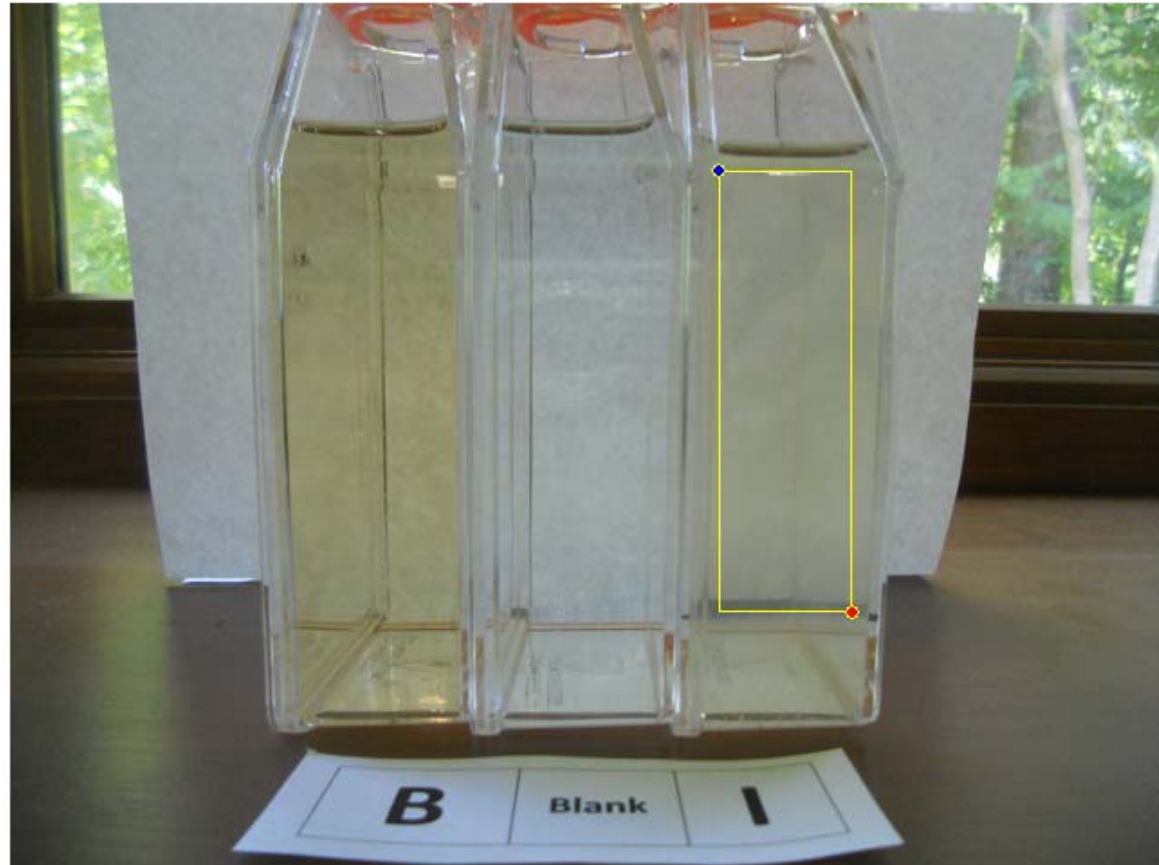
Original  Enhanced  Masked

Rectangle Tool

	Pixel Position		Adjust
	X	Y	
Start Point	393	93	
Stop Point	467	338	
Number of Pixels	18,450		

Color	Intensity [%]
Average Red	54.41
Average Green	56.68
Average Blue	49.51
Average Color	53.

Intensities of colors range from 0%, meaning none of the color is present, to 100%, when maximum color is present.



Trimmed DSCN4373.JPG is 640 by 480 pixels

Zoom In Magnification: 1.00 x Zoom Out

When zoomed in, pan around the image by using the arrow keys or hold the SHIFT key and click and drag the image.

# Rectangle Tool: I bottle

Use Spatial Analysis Tools

File Navigation Measurements Utilities Save Color Masks Apply Color Masks Help

Spatial tool features in

Select Version

Original

Rectangle Tool

Click and drag red arrows below pixel, or click a

Select Color of Tool

Start

Stop

Number

Average

Average

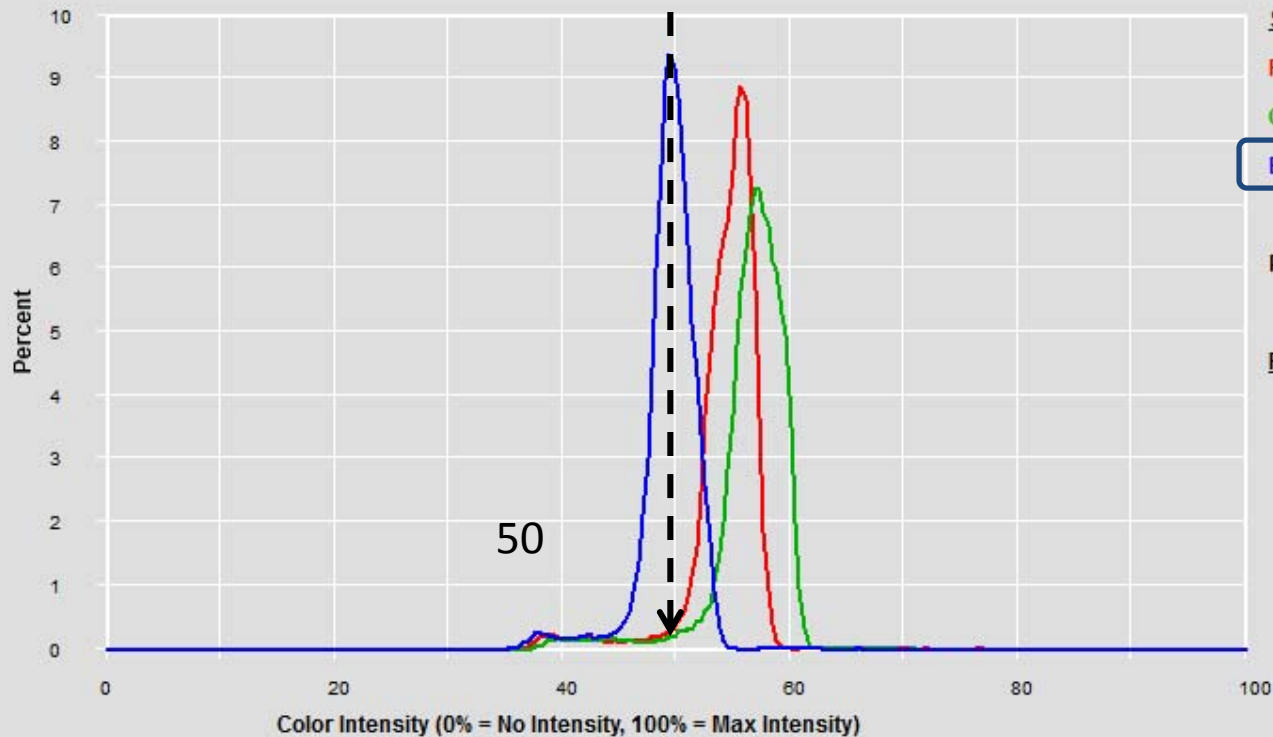
Average

Average

Intensity mean 100%

Color Histogram of Displayed Image

Color Distribution in RGB Image: Trimmed DSCN4373.JPG



Selected Area

Red: 54%

Green: 57%

Blue: 49%

Pixels: 47916

Full Image

Color histogram of currently displayed image, and, if an area is selected, the color histogram drawn in a brighter color.

If a masked image is being displayed, the color distribution of the image being masked is drawn.

Selected Area: Red  On Green  On Blue  On Average  Off

Full Image: Red  Off Green  Off Blue  Off Average  Off

Export To JPEG

Export to Text

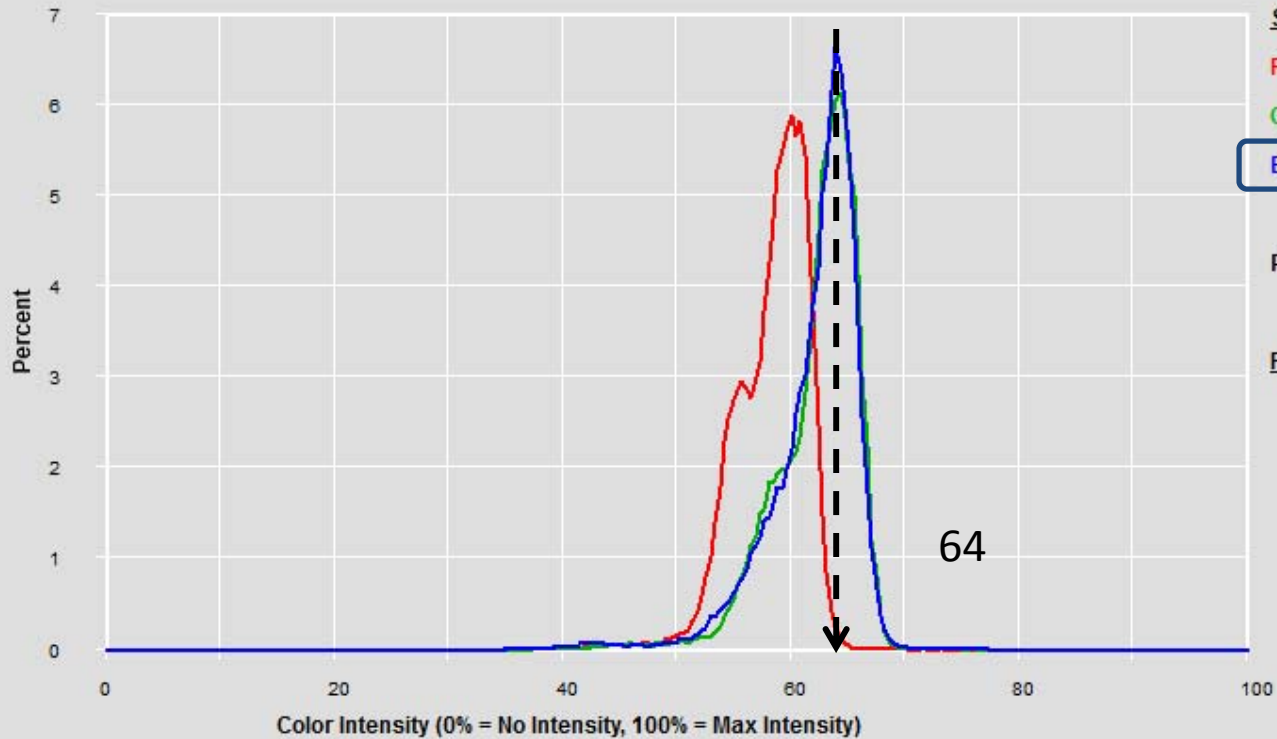
Print Graph

Close



# Rectangle Tool: Blank bottle

Color Distribution in RGB Image: Trimmed DSCN4373.JPG



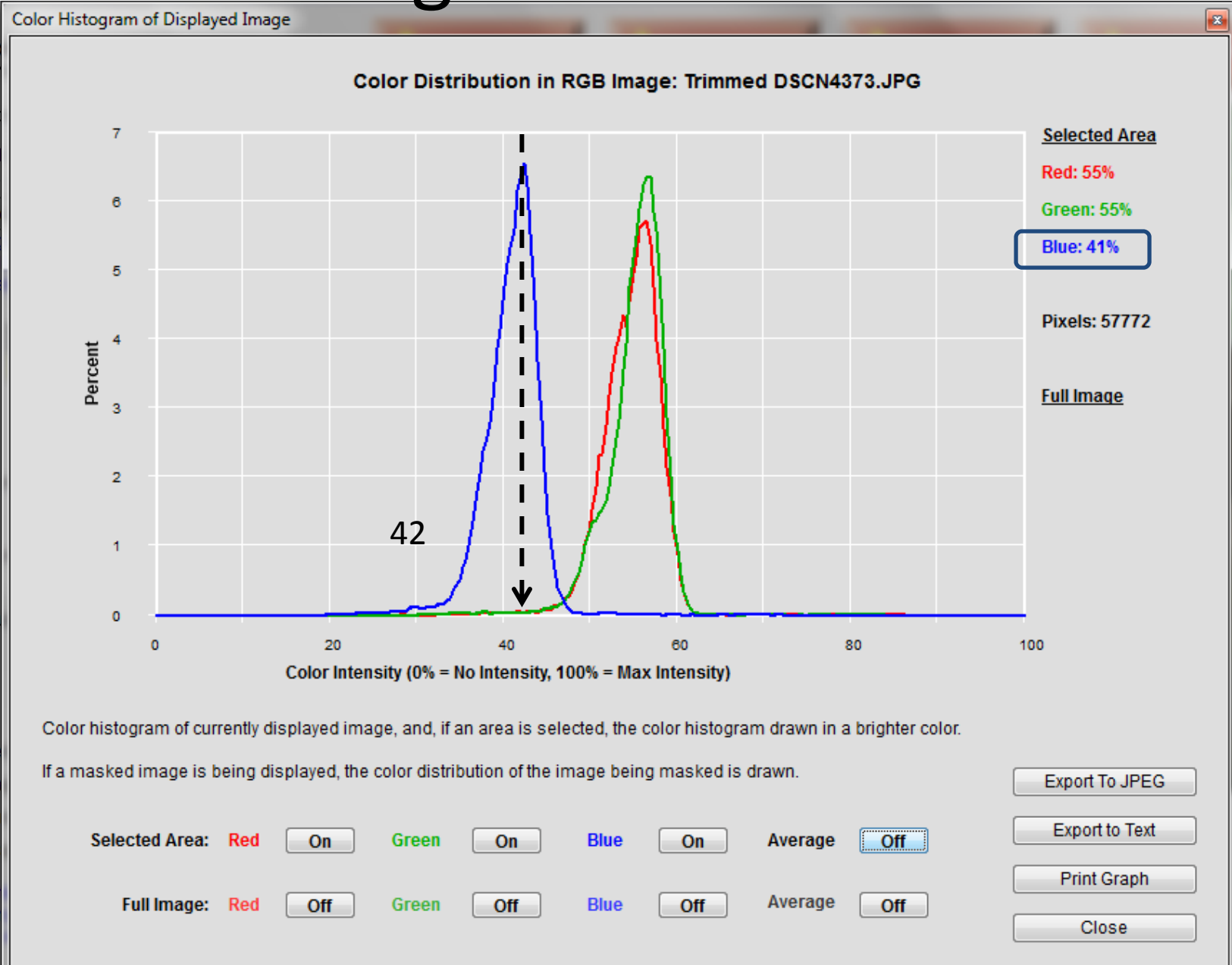
Color histogram of currently displayed image, and, if an area is selected, the color histogram drawn in a brighter color.

If a masked image is being displayed, the color distribution of the image being masked is drawn.

Selected Area:	Red	<input type="checkbox"/> On	Green	<input type="checkbox"/> On	Blue	<input type="checkbox"/> On	Average	<input type="checkbox"/> Off
Full Image:	Red	<input type="checkbox"/> Off	Green	<input type="checkbox"/> Off	Blue	<input type="checkbox"/> Off	Average	<input type="checkbox"/> Off

- Export To JPEG
- Export to Text
- Print Graph
- Close

# Rectangle Tool: B bottle



# Data Analysis

- Find location of blue peak for each rectangle on the color intensity scale
- Calculate difference between this value and the value for the blank (tap water); this is the “color lost”
- Plot “color lost” vs leaching time
  - This will show the accumulation of released colored organic matter from the leaves

# Leaching Rates

- Leaching rates from the scientific literature
- Amount released each week
  - Diminishes with time for some, accelerates for others

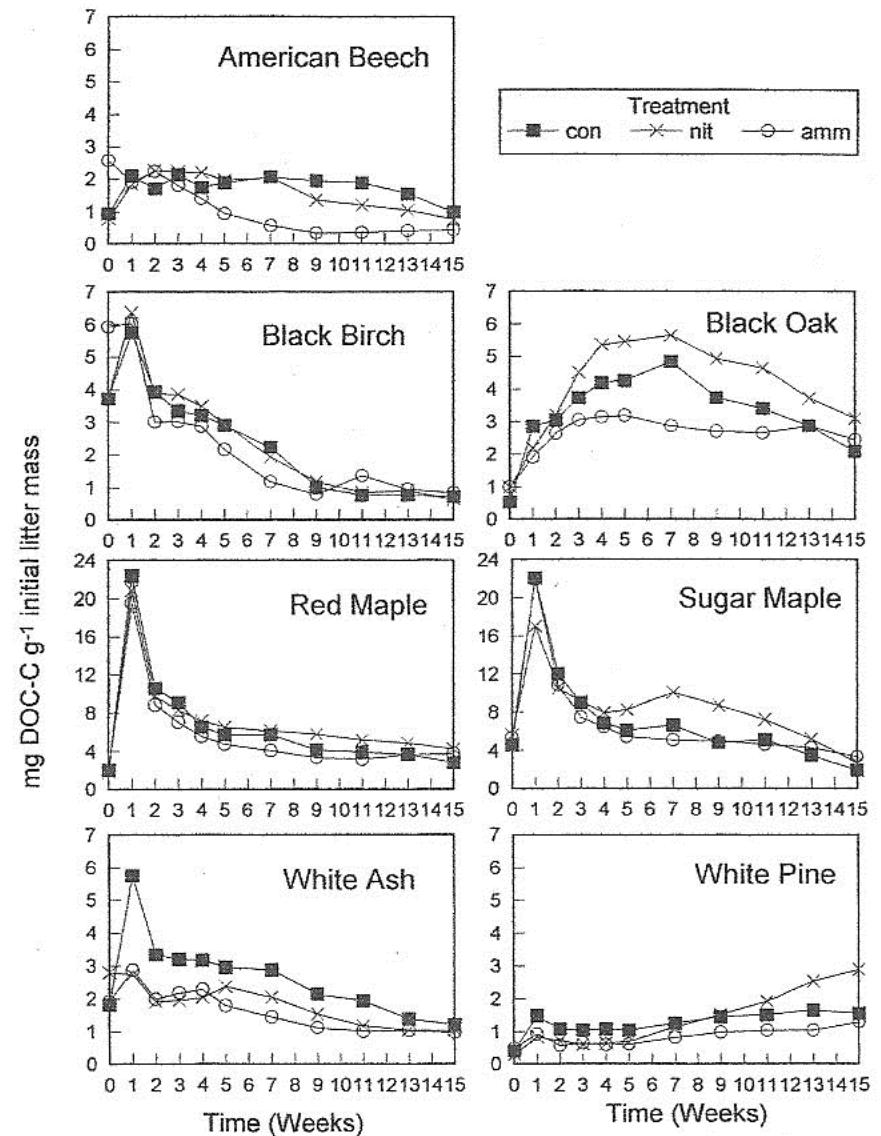
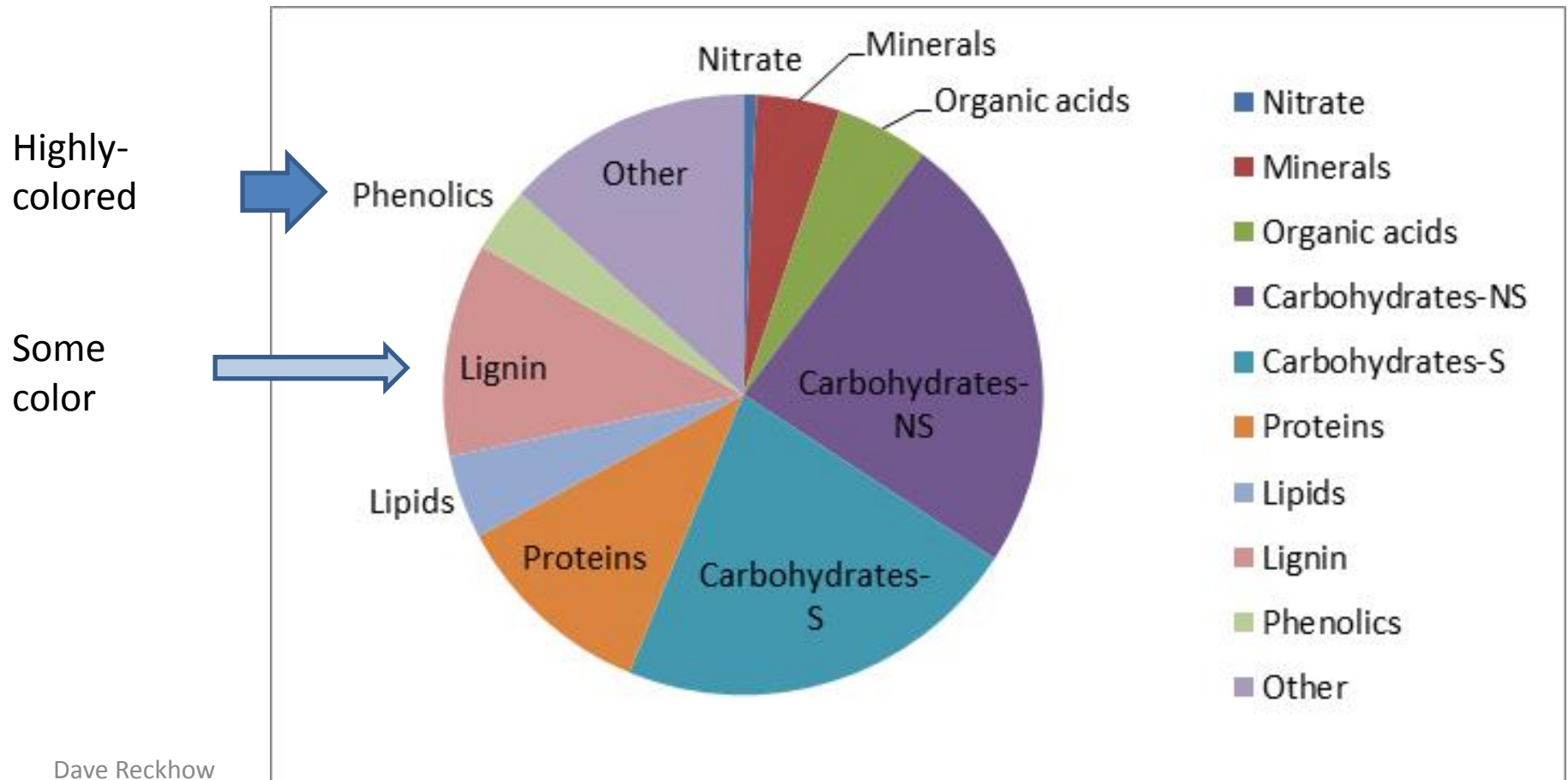


Fig. 2. Time series graphs of weekly DOC-C leached from decomposing litter over the 15 weeks as mg C g<sup>-1</sup> initial litter. Each point represents the mean of four replicate cups within each species and treatment. Note the scale differences for red and sugar maple litter. Statistical data presented in Table 3.

# Composition of an “average” leaf

- 250 g/m<sup>2</sup>/yr EABP





**TABLE 12.1 Concentration of Major Carbon Compounds in Different Plant Materials**

(Data from McClaugherty et al. 1985, Larsson and Steen 1988, Morrison 1980, Hodson et al. 1984)

	Sugars and Starch (%)	Other Solubles (%)	Cellulose (%)	Lignin (%)
Woody plants				
Foliage				
Sugar maple	7.2	37.6	43.1	12.1
Red oak	7.3	25.1	47.4	20.2
White pine	5.7	27.1	44.7	22.5
Fine roots				(Suberin)
Sugar maple	3.9	14.6	47.7	33.8
White pine	5.2	20.0	49.5	25.3
Wood				
Red maple	1.1	5.9	80.5	12.5
Hemlock bark	4.1	16.7	40.3	38.9
Herbaceous plants				
Foliage and stems				
Salt marsh grass				
Tall-form, live		34.4	52.5	13.1
Tall-form, dead		28.9	57.7	14.4
Tall-form, stems		30.3	56.0	13.7
Ryegrass stems				3-9
Leaves				2-6
Timothy stems				5-9
Leaves				3-6
Roots				
Salt marsh grass		36.2	41.6	12.2
Mixed pasture grasses		20	58	22

# Variations based on Species

- Source:
  - Terrestrial Ecosystems
    - Aber & Melillo
    - 2<sup>nd</sup> edition
    - Harcourt Academic Press

# Colored Compounds

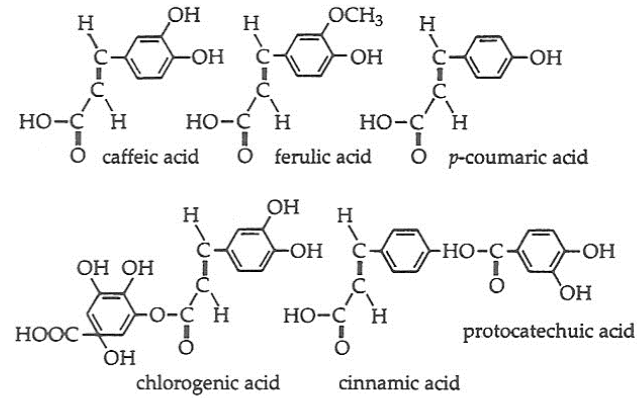
- Phenolic Acids

- Readily released, highly colored

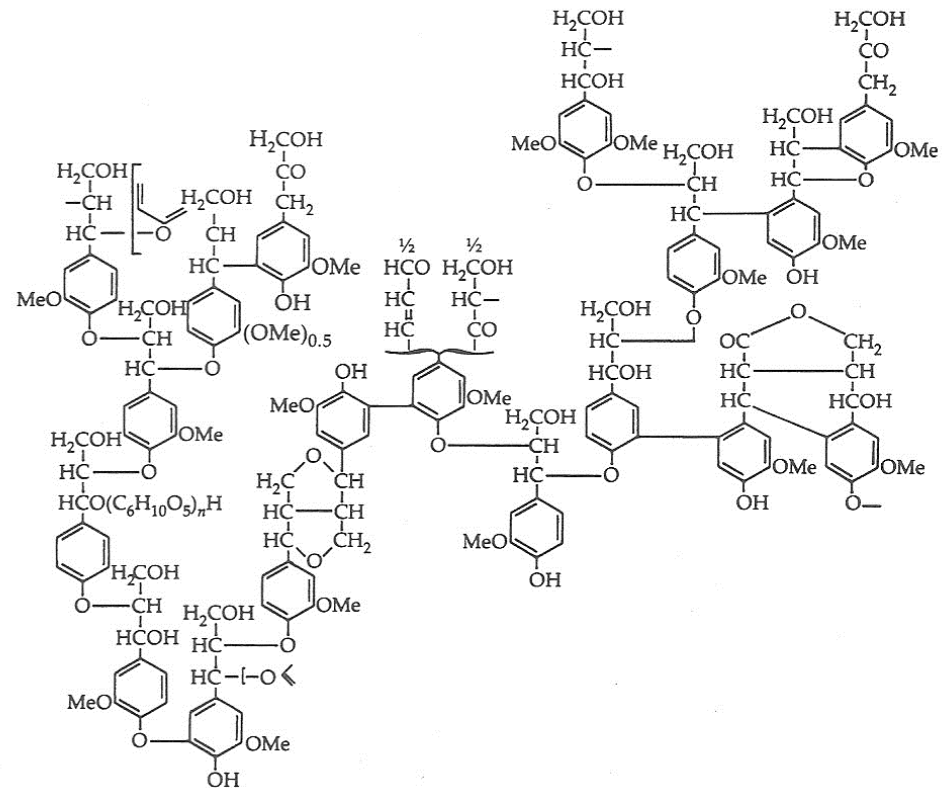
- Lignin

- Very slowly released, some color

(a) Common phenolic acids



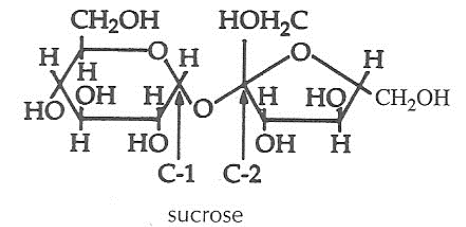
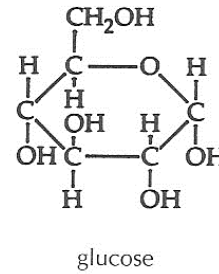
(b) Proposed subunit of a lignin molecule



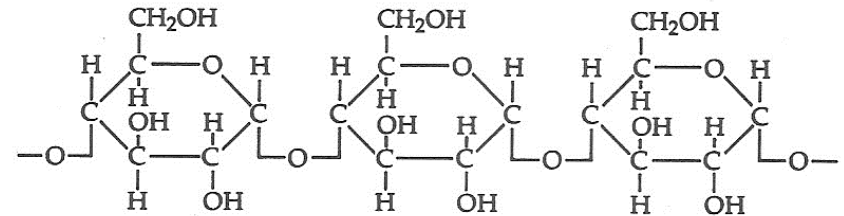
# Colorless Compounds

- Simple sugars
  - Readily released, highly biodegradable
- Starch
  - Easily released and also biodegradable
- Cellulose & Hemicellulose
  - Slow to solubilize, not easily degraded

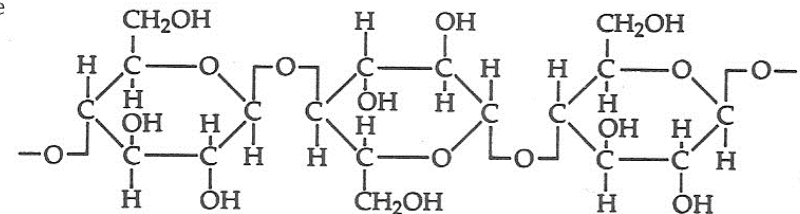
(a) Simple sugars



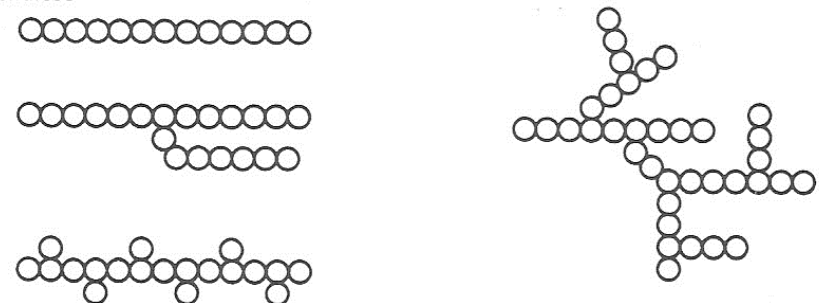
(b) Starch



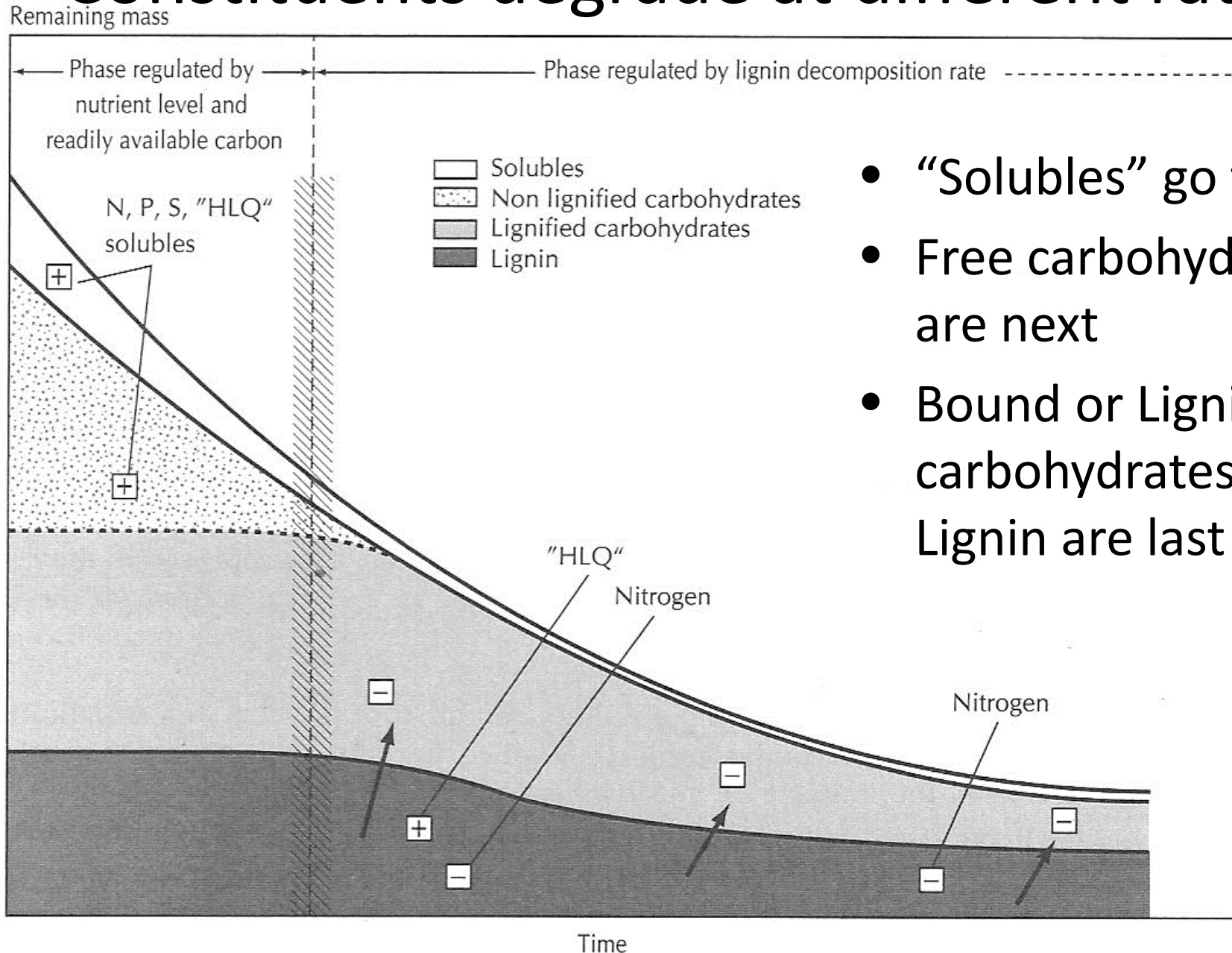
(c) Cellulose



(d) Hemicellulose



# Constituents degrade at different rates



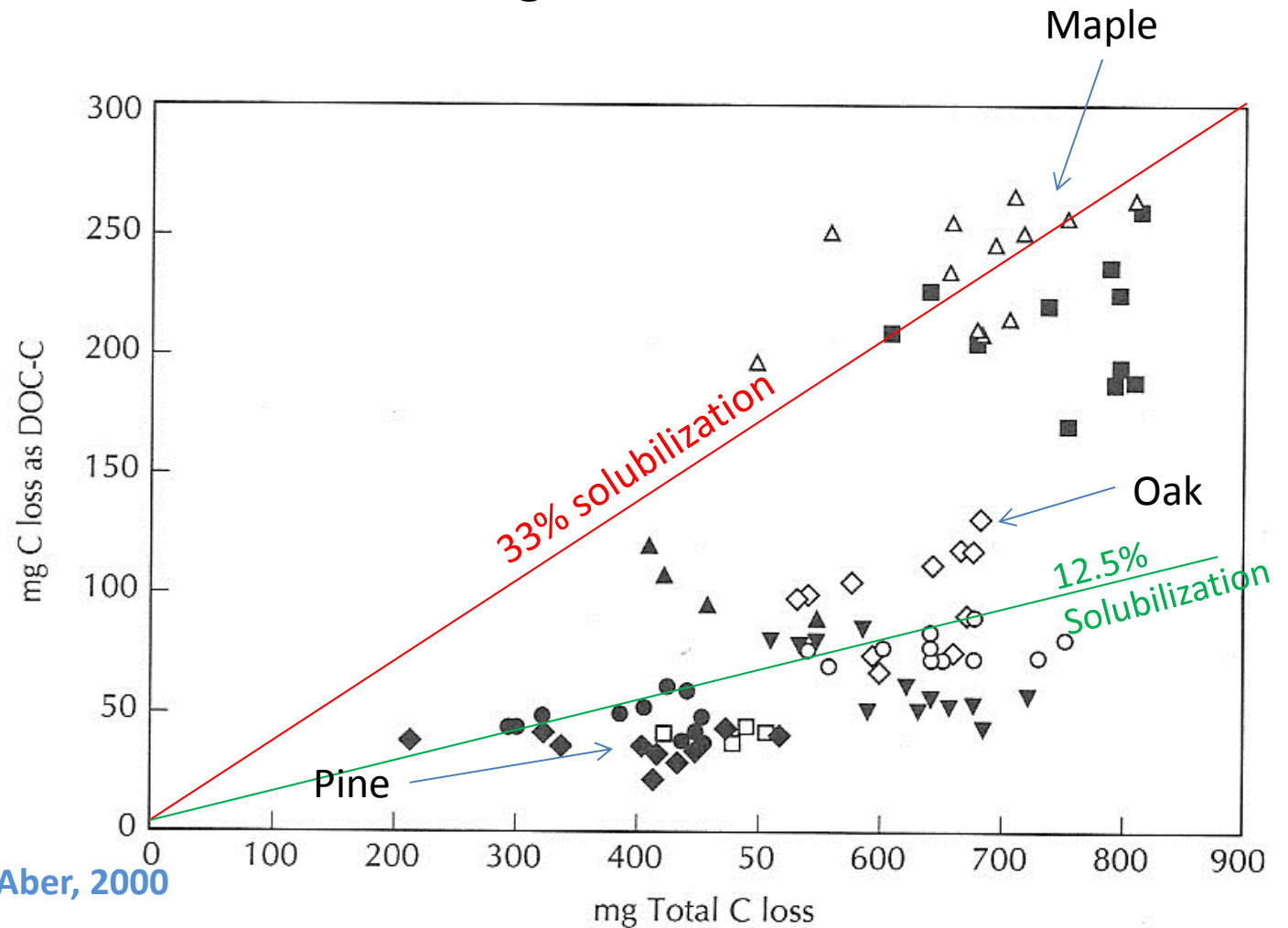
- “Solubles” go first
- Free carbohydrates are next
- Bound or Lignified carbohydrates and Lignin are last

# Solubilization vs Total Loss

- DOC-C loss versus total C loss in mg C.

Y-axis values are mean leached DOC concentration for the 15 week treatment

X-axis values are total C loss from litter.



Re-drawn from Magill and Aber, 2000



# What to Do for the Leaching Tests?

- Monday
  - Step 1: decide on plant material to use
  - Step 2: select and weigh out sufficient biomass
    - I'd recommend something between 20 and 100 grams
  - Step 3: add biomass to a plastic aquarium
  - Step 4: fill with tap water to 4 cm below top
    - This will give you a total volume of 10 Liters
- Tuesday, Wednesday, Thursday
  - Collect one photographic image of aquarium next to blank
- Friday
  - Collect final aquarium photo and begin treatment tests

# Step 1: Select Leaves

- Biomass smorgasbord

Label	Short Description	Notes
A	16 Month Aged Oak/Maple litterfall	Partially degraded from 2010 growing season, collected in March 2012
B	7 Month Aged Oak/Maple litterfall	Partially degraded from 2011 growing season, collected in June 19, 2012
C	7 Month Aged Oak on Tree	Collected from Fitzgerald Lake Conservation area on June 23, 2012 – branches that probably fell during the 10/30/2011 storm
D	7 Month Aged White Pine	Collected from Fitzgerald Lake Conservation area on June 23, 2012 - branches that probably fell during the 10/30/2011 storm
E	Fresh Oak	Collected from freshly cut branches on June 23, 2012
F	Fresh Cherry	Collected from freshly cut branches on June 23, 2012
G	Fresh Mulberry	Collected from freshly cut branches on June 23, 2012
H	Fresh Bittersweet	Harvested on June 23, 2012, whole branches
I	Fresh Goldenrod	Harvested on June 23, 2012, whole plants

# Extra Topic: Beer's Law

- Concentration of a pure solution of an absorbing compound is directly proportional to the logarithm of the light intensity for experimental (I) divided by the light intensity for the blank (I<sub>0</sub>)
  - This is the "Absorbance"

$$\text{Absorbance} = -\text{Log}\left(\frac{I}{I_0}\right)$$

Fixed value
Fixed value

$$\text{Absorbance} = \text{concentration} \times \text{pathlength} \times \text{absorptivity}$$

Sample	Blue Intensity (I)		I/I <sub>0</sub>	Absorbance =-LOG(I/I <sub>0</sub> )
	observed	lost		
blank	64	0	1	0
I	50	14	0.781	0.107
B	42	22	0.656	0.183