Bridging research and teaching:

Bringing environmental research-like experiences into the undergraduate analytical teaching laboratory

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Instrumental Analysis at Seattle U

CHEM 426 Instrumental Analysis 5
Theory and techniques of instrumental methods representative of spectrometric, electroanalytical, and chromatographic techniques. Two lecture and two four-hour laboratory periods per week. Prerequisites: CHEM 319, 361. (spring)

Enrollment is typically 10 - 20 students

- split between juniors and seniors
- mostly chemistry/biochemistry majors
- occasional biology major
- students ideally work in pairs

Instrumental Analysis at Seattle U

Instrumental Analysis at Seattle U has sometimes been taught in close collaboration with SU's senior-level ecology course. We try to focus on labs of local/ecological/environmental interest.

<u>The labs:</u>

- IR: xylenes quantification
- fluorescence: Stern-Volmer quenching
- AAS: lead in soils (request from People for Puget Sound)
- GC-MS: terpenes in tree resins (Langenhan group research)
- LC-MS-MS: pesticides analysis (Latch group research)
- UV and HPLC: pollutant photochemistry (Latch group research)

Lead analysis: legacy of the Asarco smelter

- Asarco Company had run a copper smelter out of Tacoma that emitted copious amounts of mercury and lead to the atmosphere

- Monitoring the legacy and continuing impact of mercury and lead in the path of the smelter plume is of concern to local community members/ organizations (this experiment started as a service-learning project with People for Puget Sound)

Can we contribute to the monitoring of Pb?

Focus areas:

- Vashon Island (because it is near the smelter and directly in the plume's major pathway)
- Seattle University area (because there is currently a lack of data and it is relevant to the students' interests)

Lead analysis by atomic absorption spectroscopy

In the field:

- Students collect soil samples from Vashon Island and different sites in Seattle

In the lab:

- Students develop an analytical method (based on an established EPA method)
- Create calibration curves
- Quantify lead
- Perform spike-recovery experiments
- Determine LODs

Sample student data: lead at Seattle sites

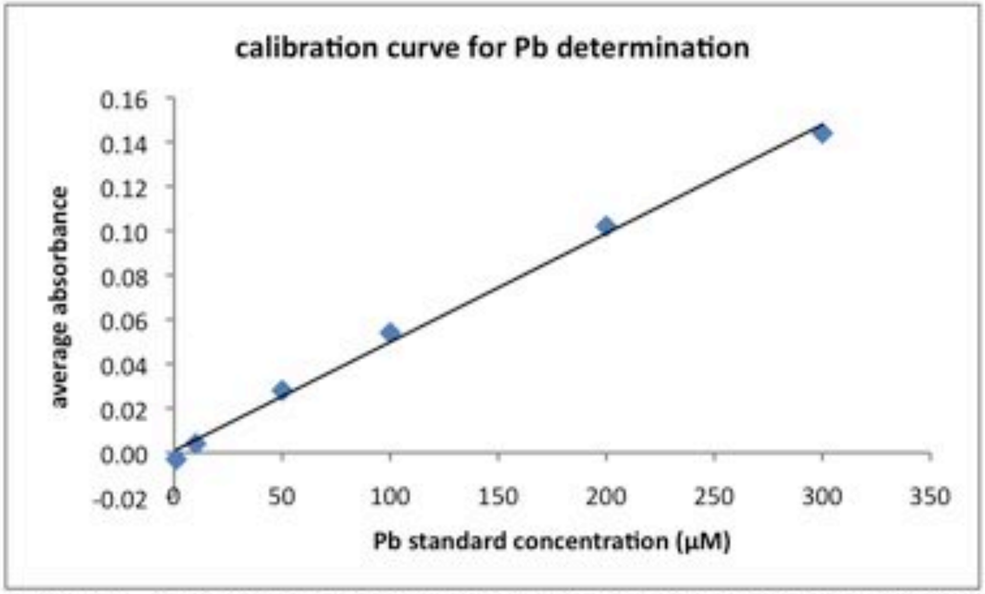


Figure 1. Calibration curve for the AAS determination of lead ($y=0.0005x+0.0009$; $R^2=0.9958$).

Table 1. AAS data for the quantitative analysis of lead in various soil samples.

Location	Coordinates	Pb Concentration (ppm w/w)
Madrona Playfield	N47°36'39.6" W122°17'23.7"	39.2
Bailey Gatzert	N47°36'00.9" W122°18'53.7"	42.4
Volunteer Park	N47°36'49.3" W122°19'00.3"	26
Cal Anderson Park	N47°36'59.4" W122°19'06.7"	13.3
Lowell Elementary	N47°37'32.7" W122°19'05.9"	19
Cal Anderson Park	N47°37'04.6" W122°19'09.3"	28.7
Bellarmine	N47°36'33.4" W122°19'03.5"	5.6
Volunteer Park	N47°37'43.7" W122°19'04.2"	92.7
Seattle Central Community College	N47°36'56.3" W122°19'16.4"	62.5

Conifer defense against pine beetles

- A pine beetle outbreak is currently threatening large areas of North America.
- Conifers' primary defense against attack is resin.
- Pine resin is comprised primarily of monoterpenes and diterpene acids.
- Preliminary results suggest that monoterpene levels can influence bark beetle attack rates and success.
- This study aims to quantify monoterpene levels in conifers at different elevations before & after predation.

Terpenes found in conifer resin

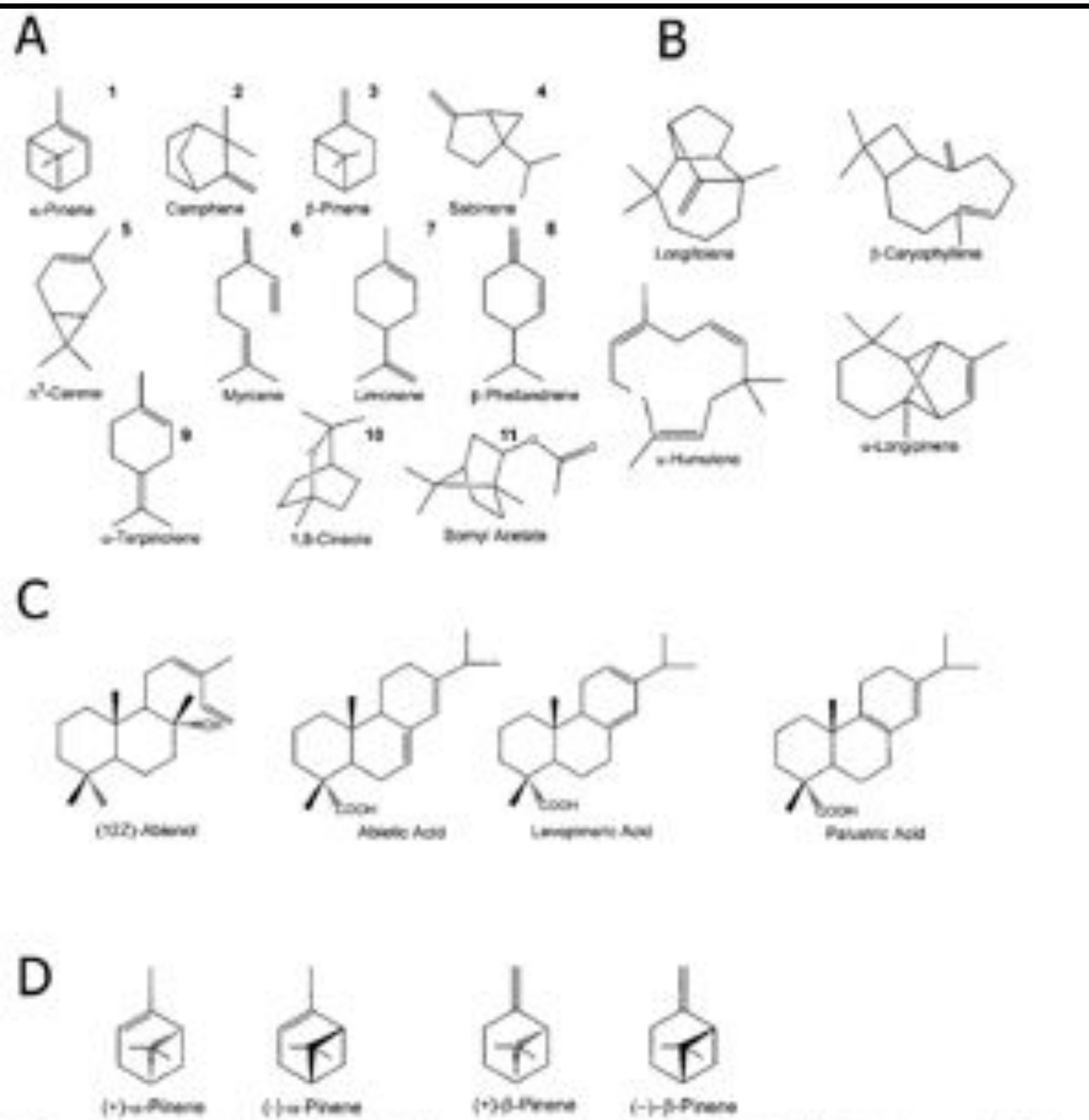


Figure 1. Representative structures of terpenoids of Norway spruce (*Picea abies* L. Karst). A and D, Monoterpenes (10 carbon atoms). B, Sesquiterpenes (15 carbon atoms). C, Diterpene resin acids (20 carbon atoms). Monoterpenes are numbered corresponding to peak numbers in Figure 9.

Martin et al. *Plant Physiology* **2002**, *129*, 1003-1018.

Sample student data: limonene calibration

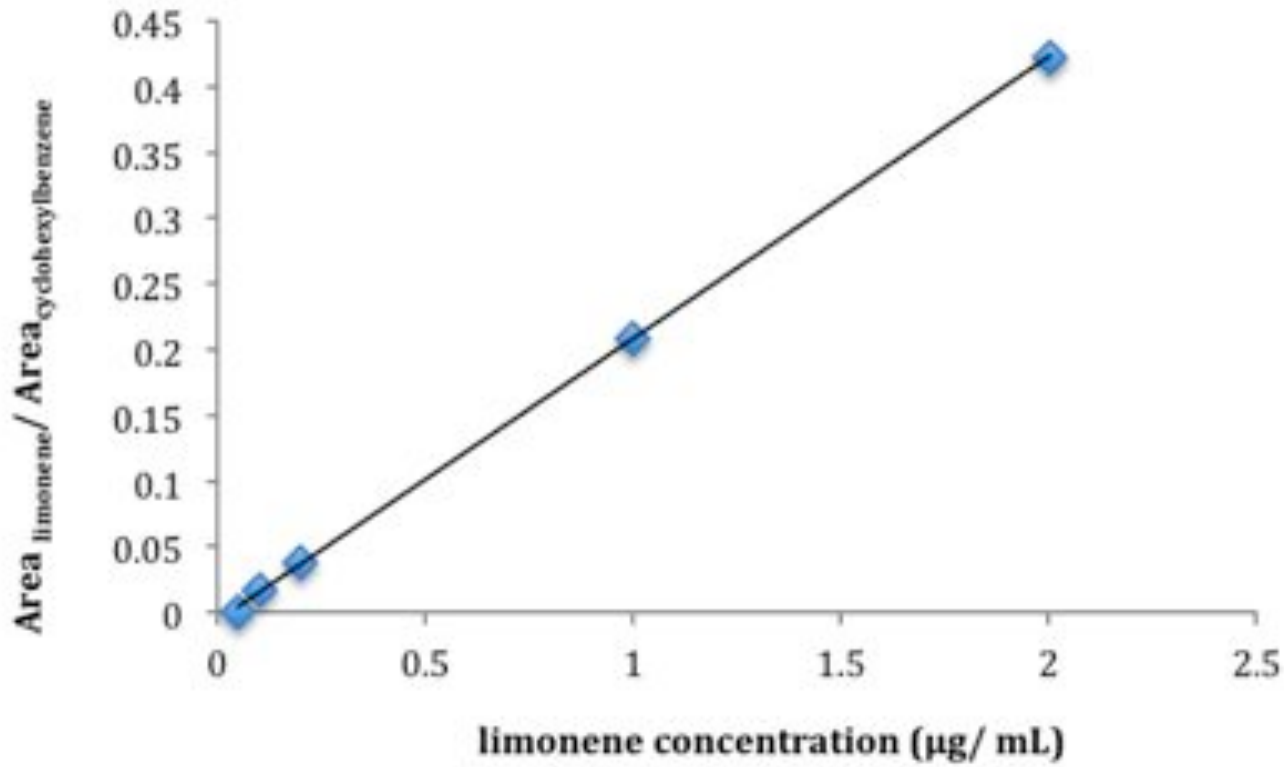


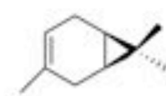
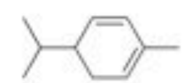
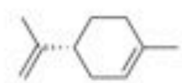
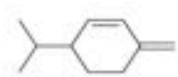
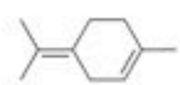
Figure 1. Calibration curve for the GC-MS determination of limonene using cyclohexylbenzene as internal standard (y=0.2148x-0.0066; R^2=0.99979)

Sample student data: identification of terpenes

Table 1. GC-MS data for the quantitative analysis of terpenes using cyclohexylbenzene as an internal standard

Sample	Retention time (min)	Area terpene (Total ion count)	Terpene
LR 10	6.178	279483	α-pinene
	6.745	98276	sabinene
	7.283	951741	(+)-3-carene
	7.581	1491762	(+)-limonene
	8.409	491764	terpinolene
LR 16	6.177	857474	α-pinene
	6.746	2600900	sabinene
	6.846	330291	β-pinene
	6.926	120793	myrcene
	7.284	3875840	(+)-3-carene
	7.389	102111	α-phellandrene
	7.580	2341989	(+)-limonene
	7.996	109494	β-phellandrene
	8.342	41779	terpinolene
LR 21	6.185	507335	α-pinene
	6.853	148639	β-pinene
	6.932	69809	myrcene
	7.590	4900556	(+)-limonene
LR 47	6.176	177523	α-pinene
	7.282	865712	(+)-3-carene
	7.579	311292	(+)-limonene
	8.408	203989	terpinolene
LR 57	6.179	1703641	α-pinene
	6.440	51228	camphene
	6.745	1046285	sabinene
	6.848	331230	β-pinene
	6.927	211998	myrcene
	7.287	6539980	(+)-3-carene
	7.392	209173	α-phellandrene
	7.582	2903751	(+)-limonene
	7.997	168454	β-phellandrene
	8.409	2395575	terpinolene

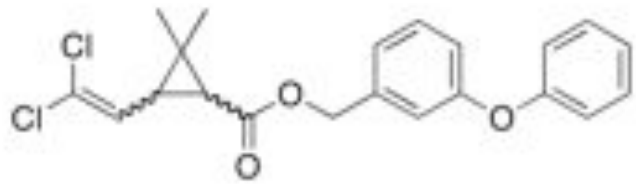
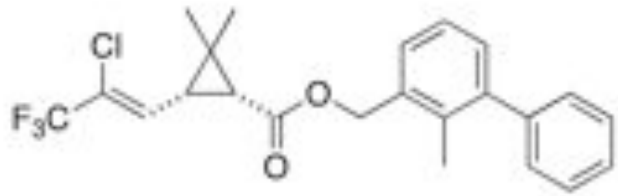
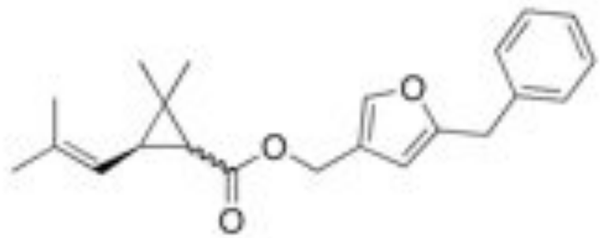
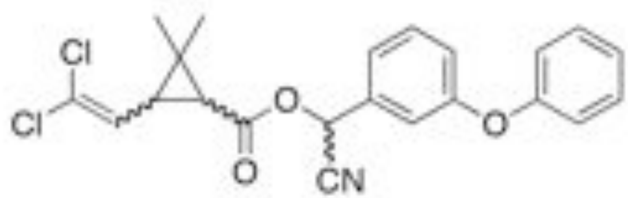
Table 2. Structures of terpenes found using GC-MS in unknown resin samples

Terpene	Structure
α-pinene	
Camphene	
Sabinene	
β-pinene	
Myrcene	
(+)-3-carene	
α-phellandrene	
(+)-limonene	
β-phellandrene	
Terpinolene	

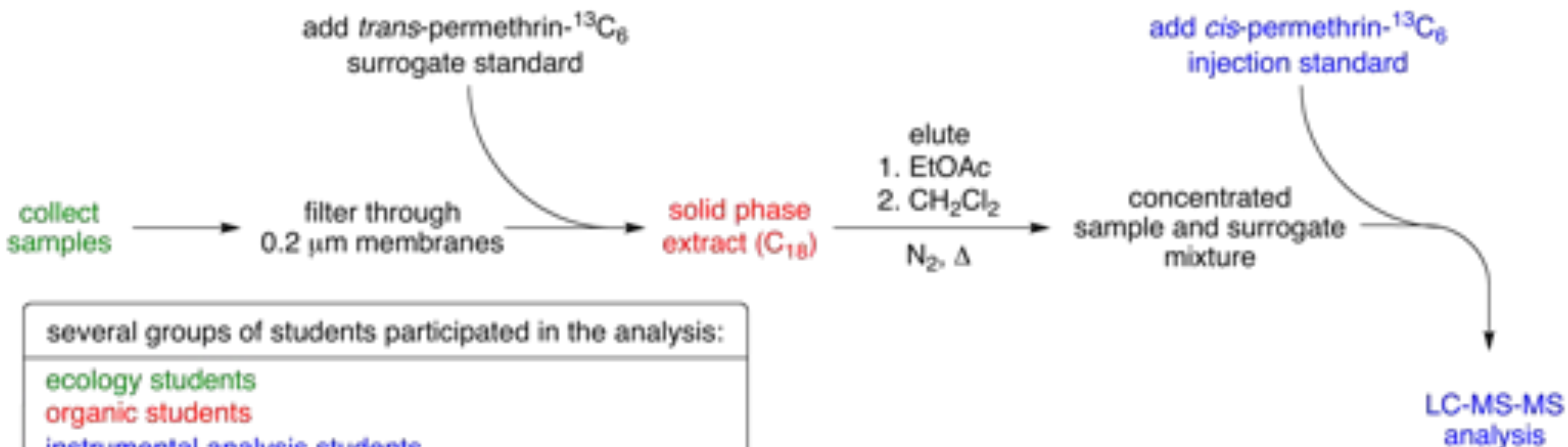
Duwamish River: a local EPA Superfund site

Emerging contaminants: pyrethroids

Found in over 3,500 commercial products

Compound name	Compound structure
permethrin	
bifenthrin	
resmethrin	
cypermethrin	

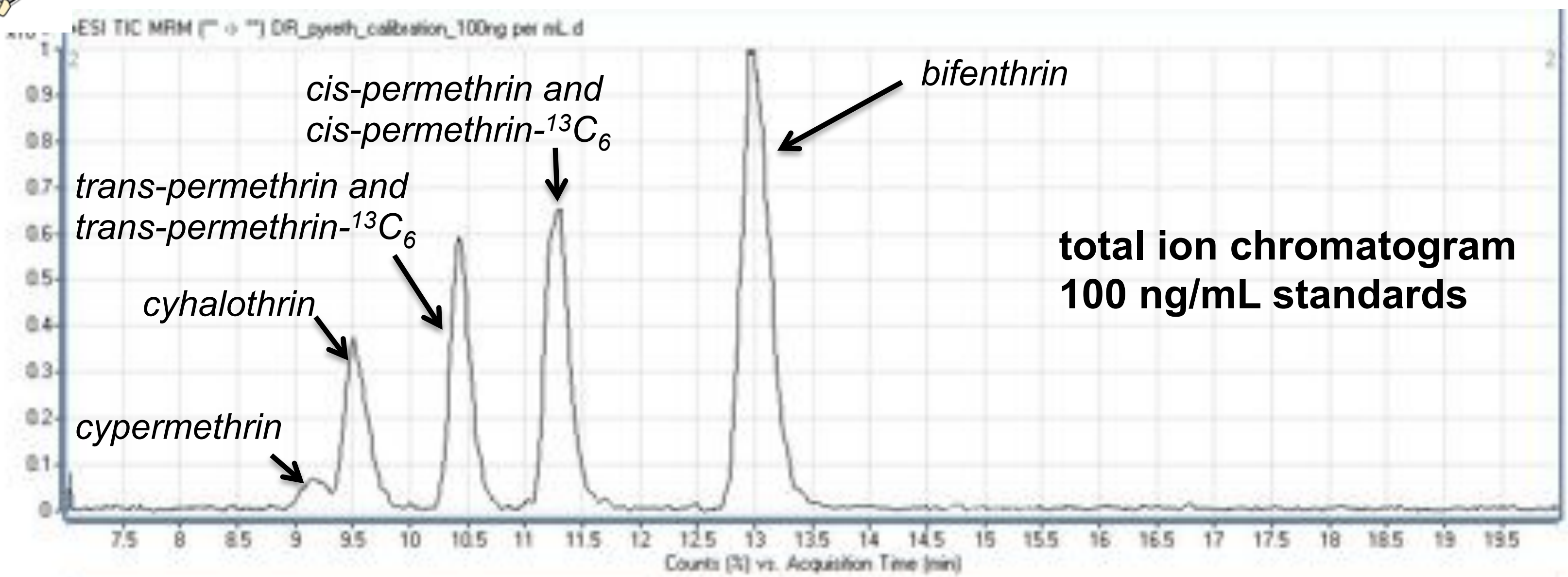
Summary of pyrethroids analysis

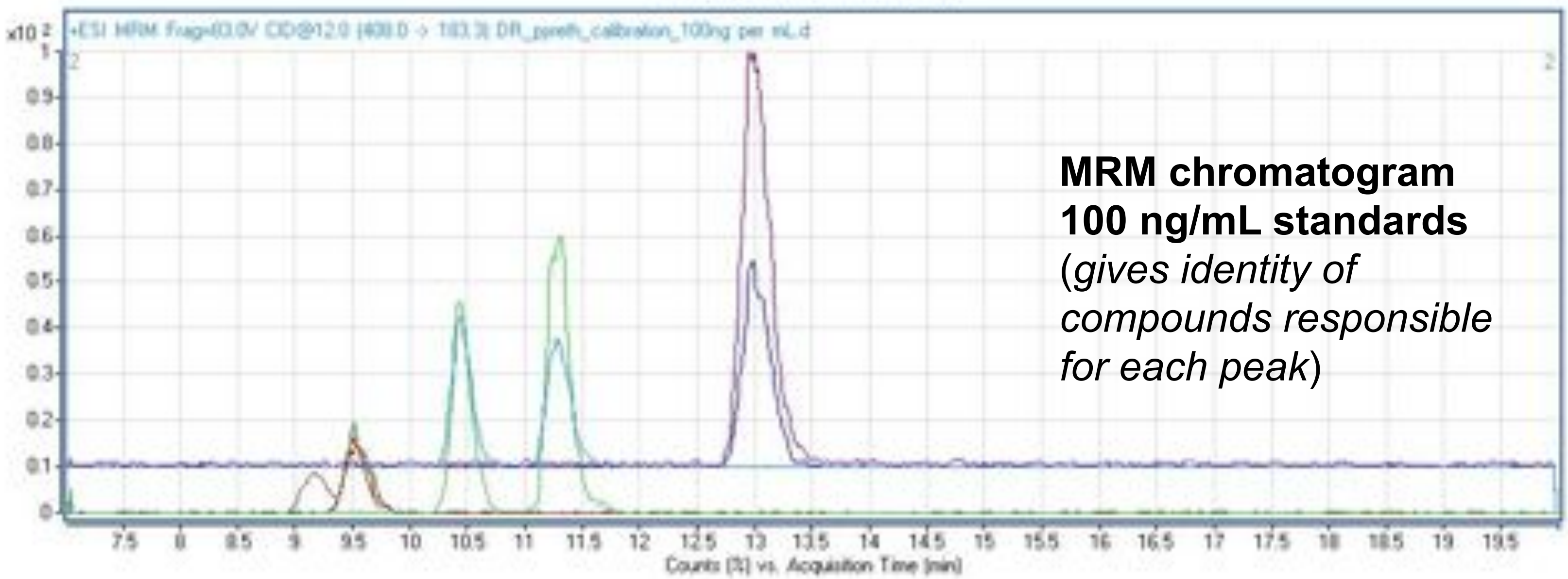


several groups of students participated in the analysis:

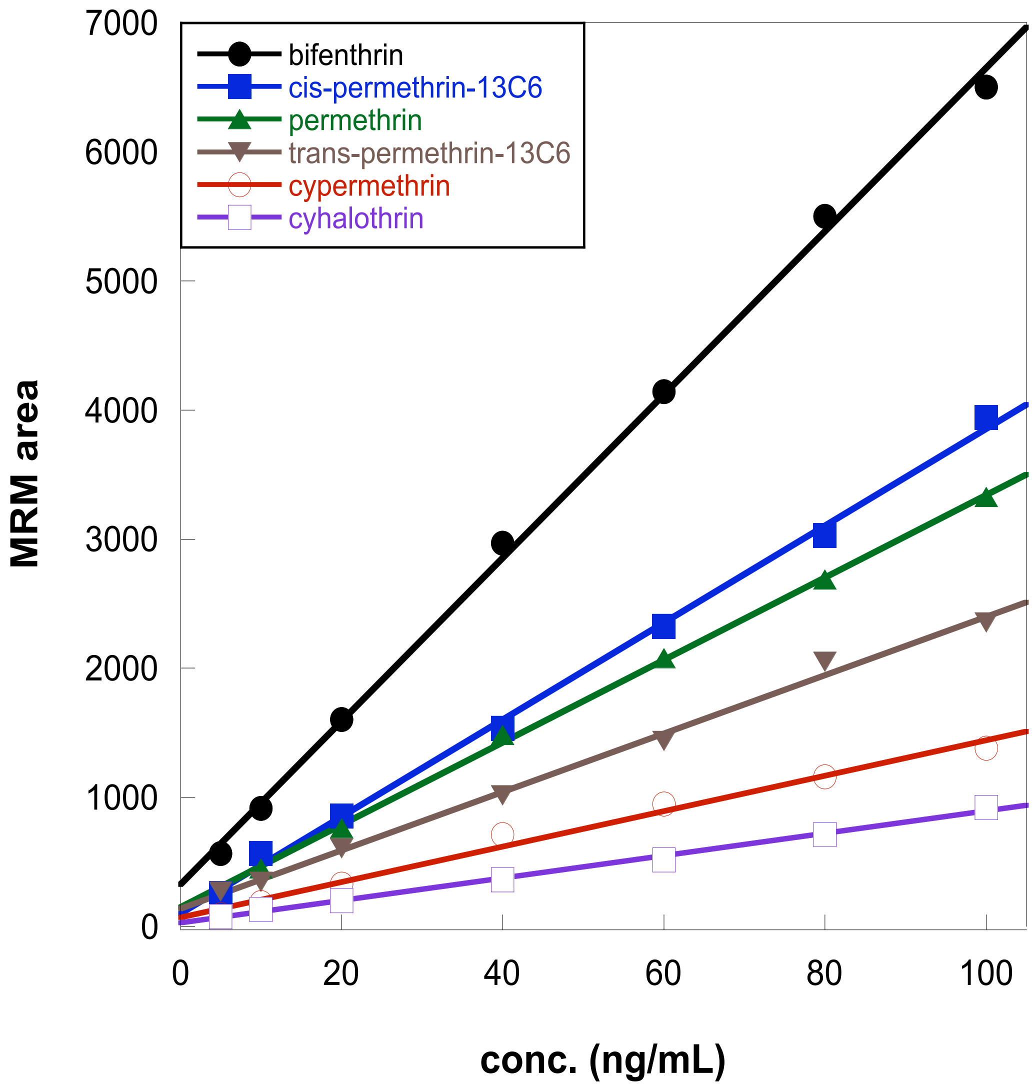
- ecology students
- organic students
- instrumental analysis students
- research groups

LC-MS/MS: output from standards





Sample student data: pyrethroids calibration

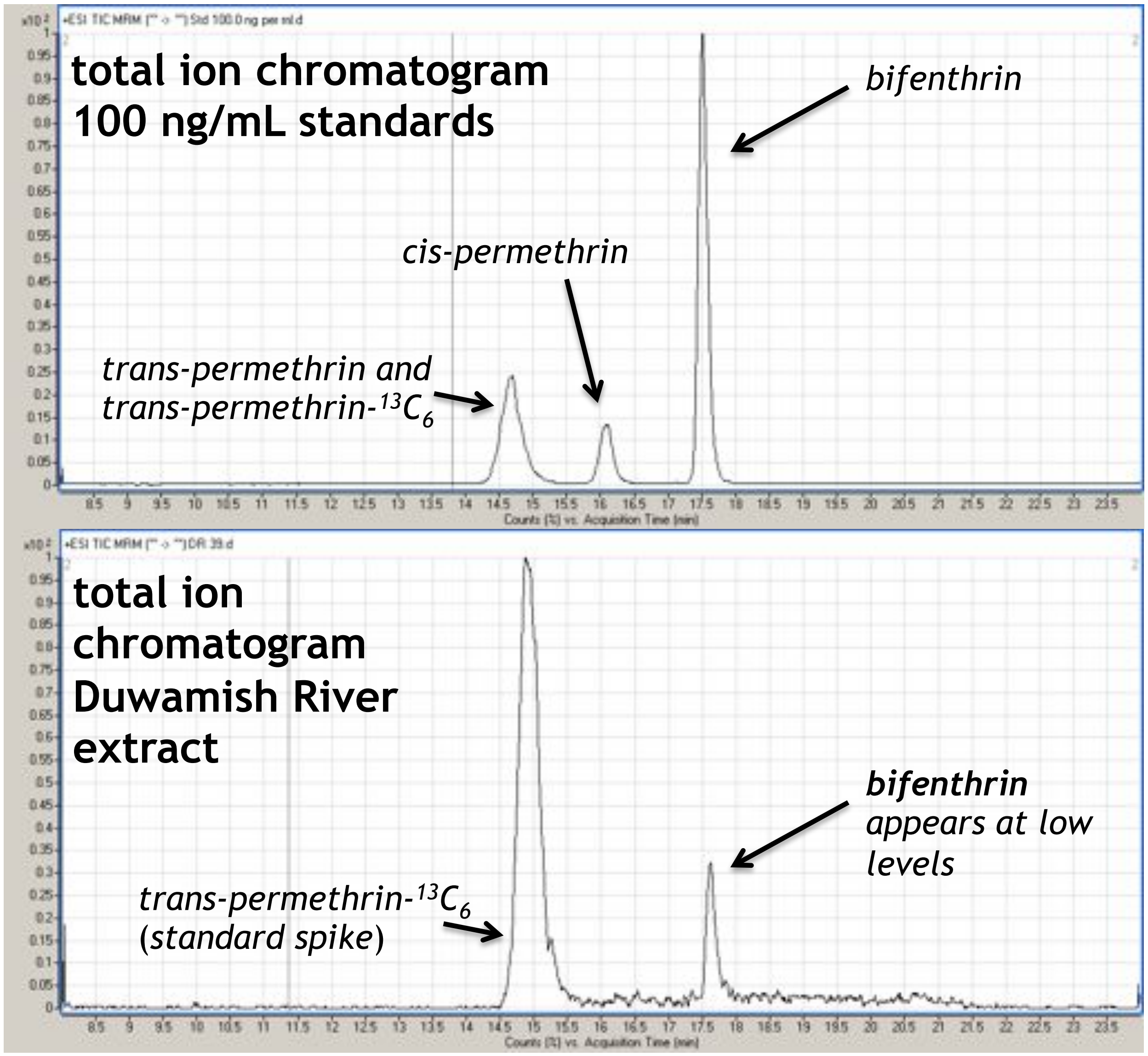


The LC-MS-MS method displays great sensitivity and LODs

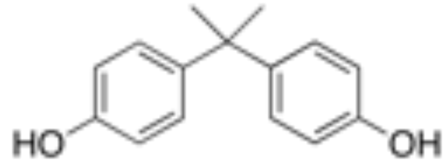
Recoveries of better than 90% are achieved

We are capable of measuring pyrethroids in water, sediment, and tissue samples

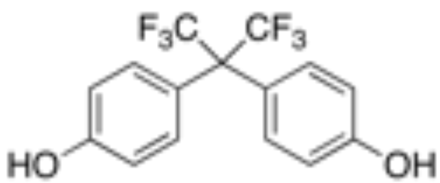
Bifenthrin in Duwamish River water



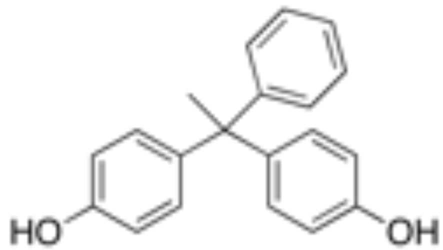
Bisphenols in the environment



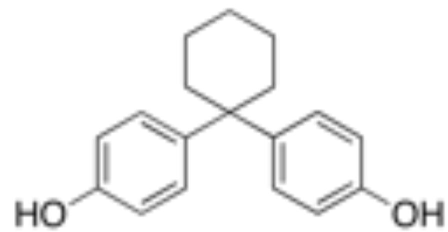
bisphenol A
"BPA"



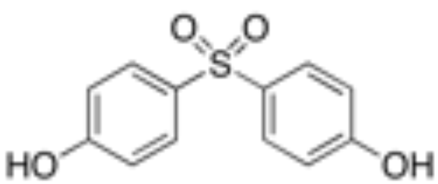
bisphenol AF
"BPAF"



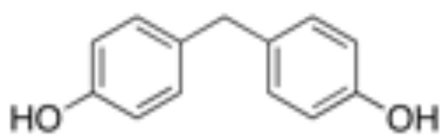
bisphenol AP
"BPAP"



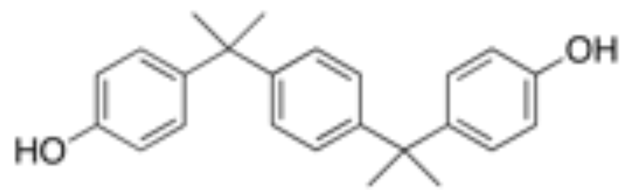
bisphenol Z
"BPZ"



bisphenol S
"BPS"

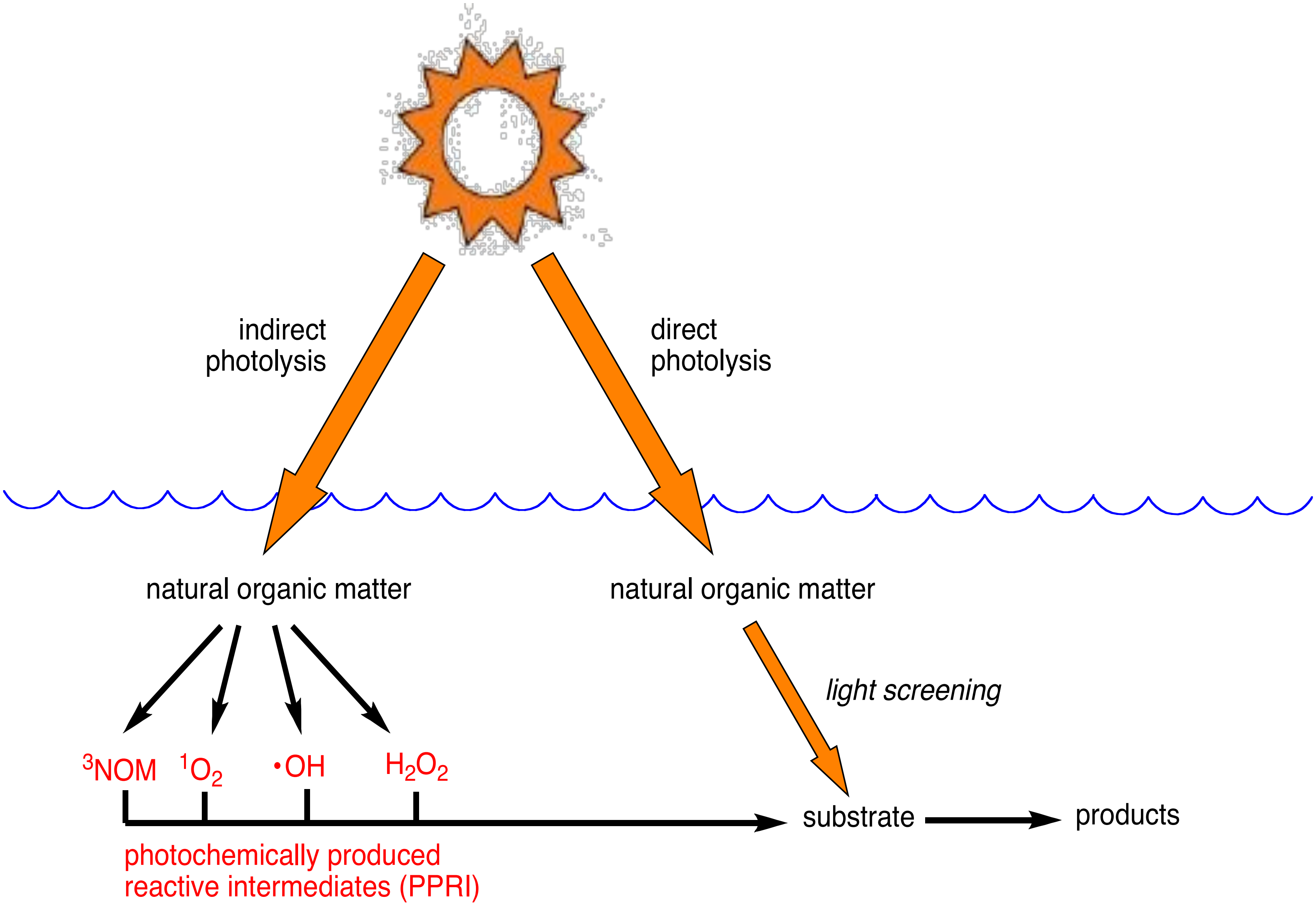


bisphenol F
"BPF"



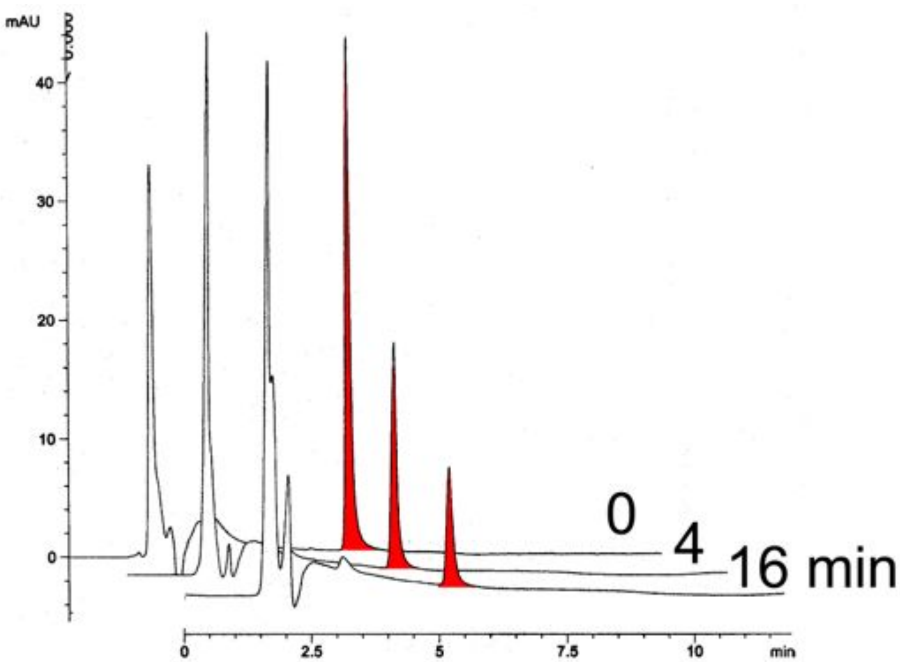
bisphenol P
"BPP"

Photochemical behavior of pollutants

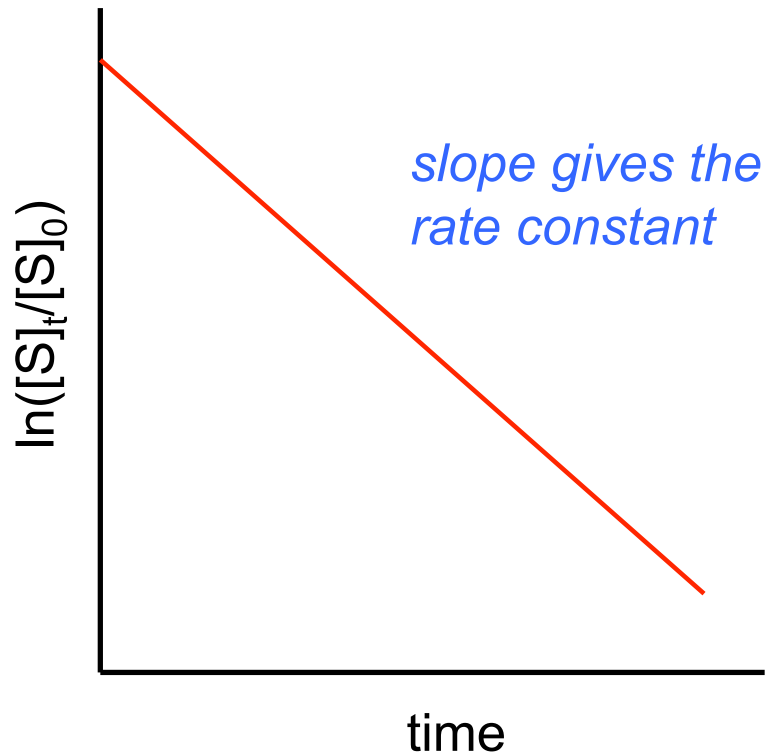


Measuring photochemical kinetics

substrates quantified by peak area



degradation data treated according to pseudo-first order kinetics



Sample student data: BPZ UV analysis

UV results were used in developing HPLC methods and for interpreting photochemistry data

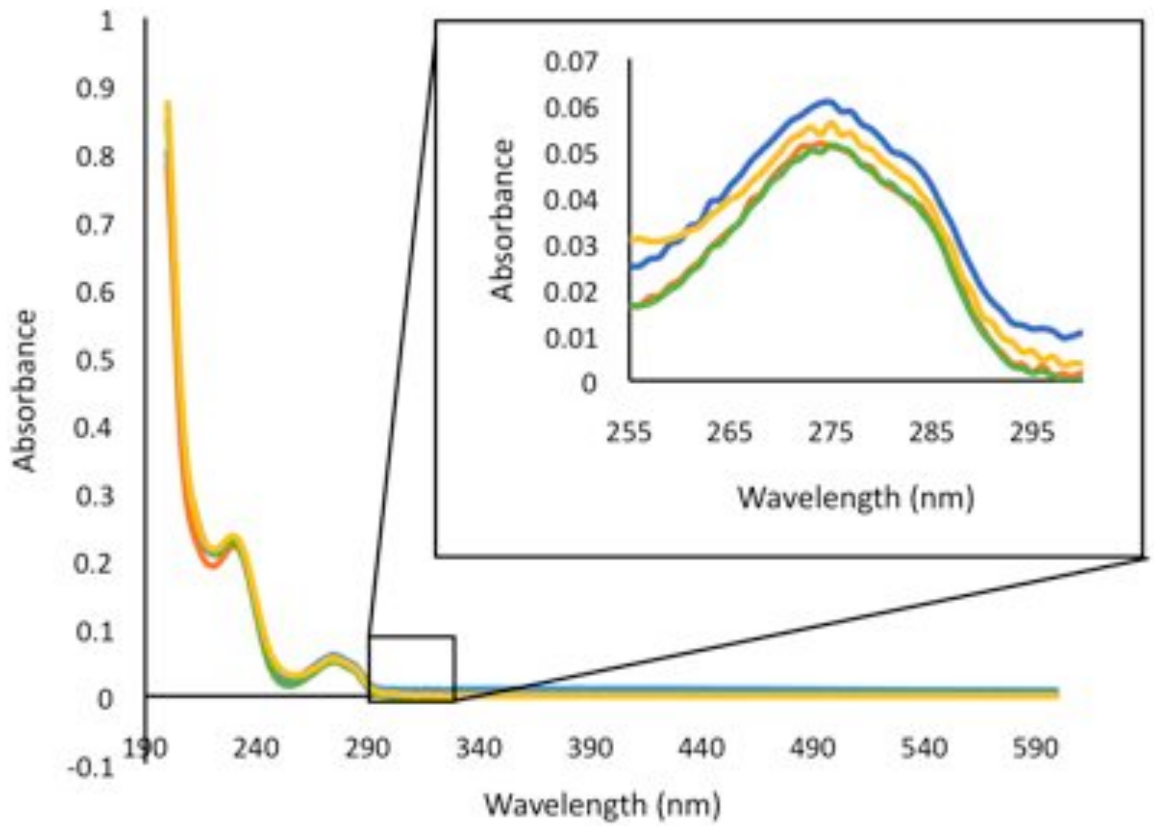


Figure 1. Observed absorbance spectrum of BPZ at various pH levels with magnified inset of wavelengths that insersect with visible spectrum. Blue = pH 6; Red = pH 7; Green = pH 8; Yellow = pH 9

Sample student data: BPZ photochemistry

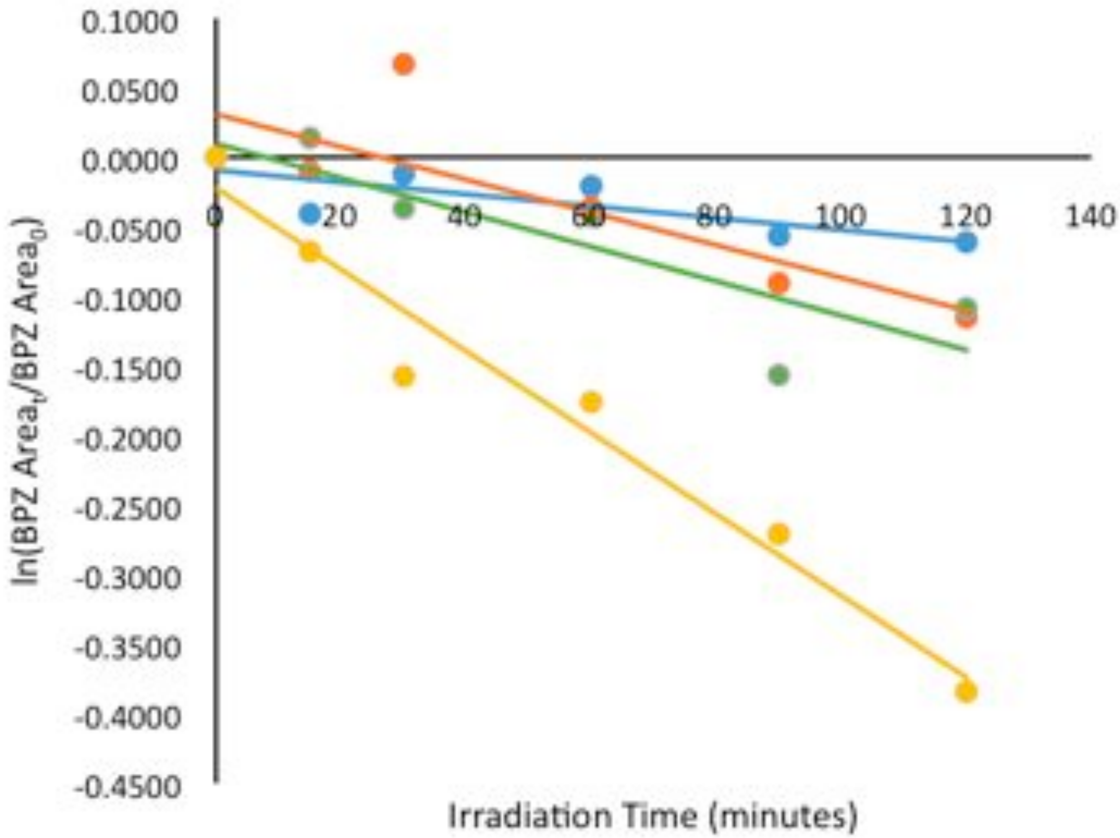
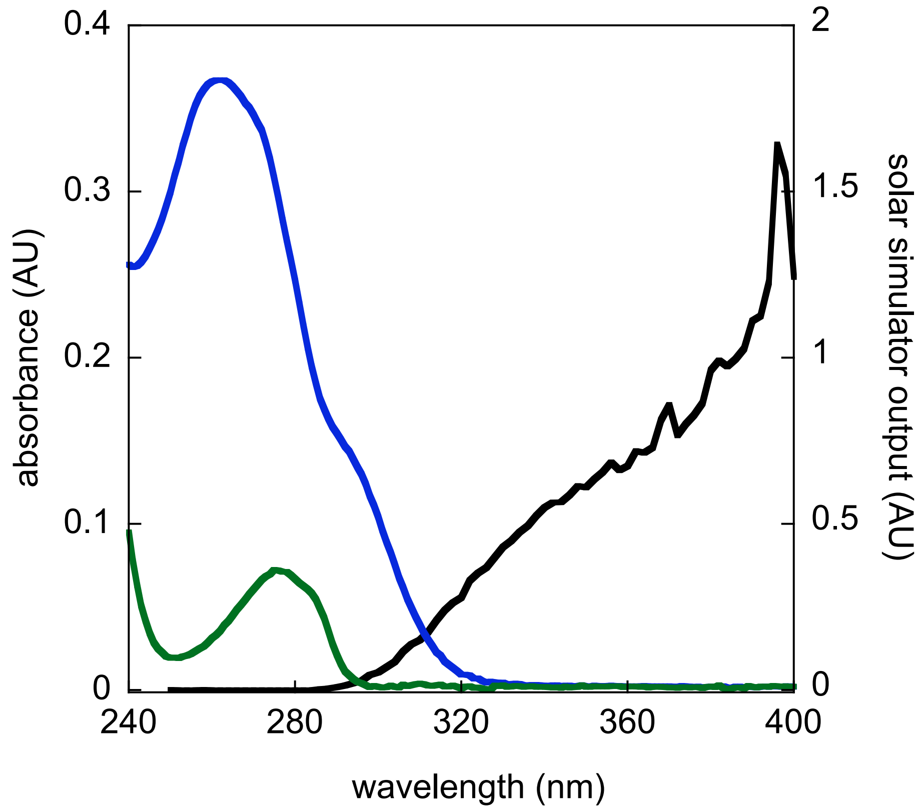
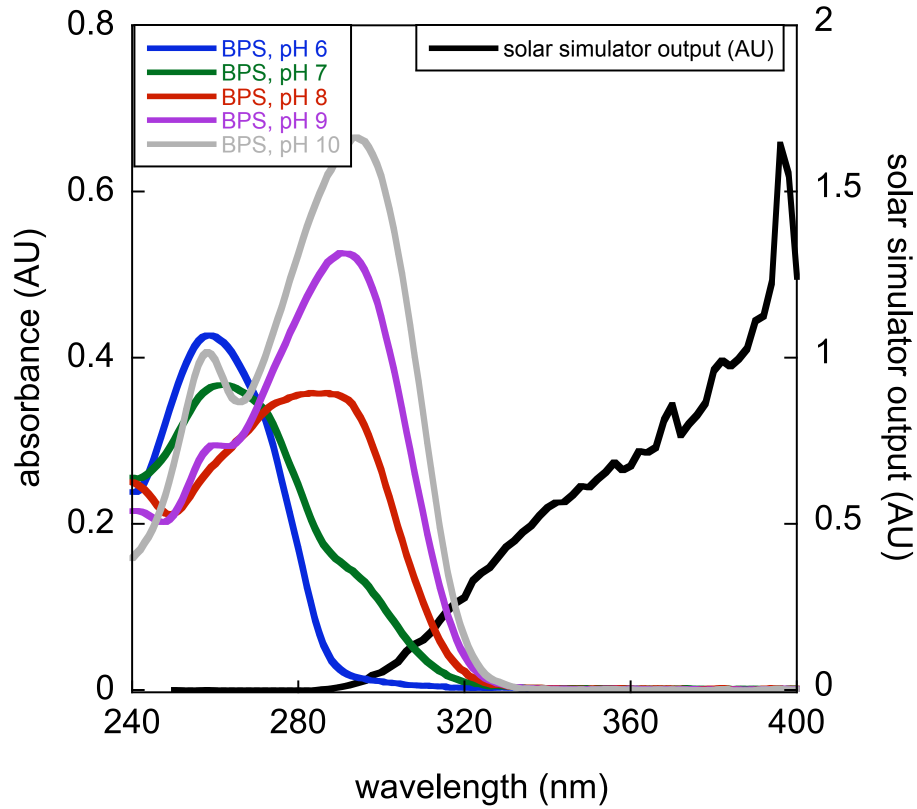


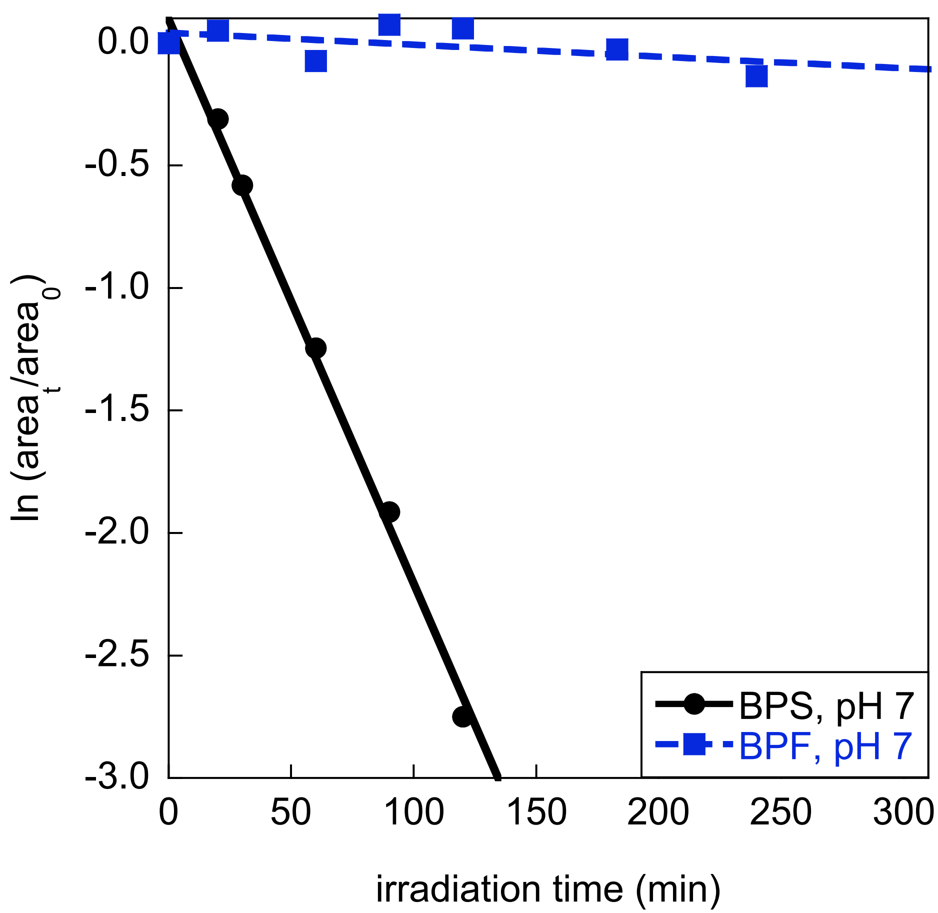
Figure 2. Degradation of BPZ under direct and indirect photolysis conditions. Blue = Direct, pH 7; Red = Direct, pH 8; Green = Indirect, 10 ppm SRFA, pH 7; Yellow = Indirect, 10 ppm PLFA, pH 7

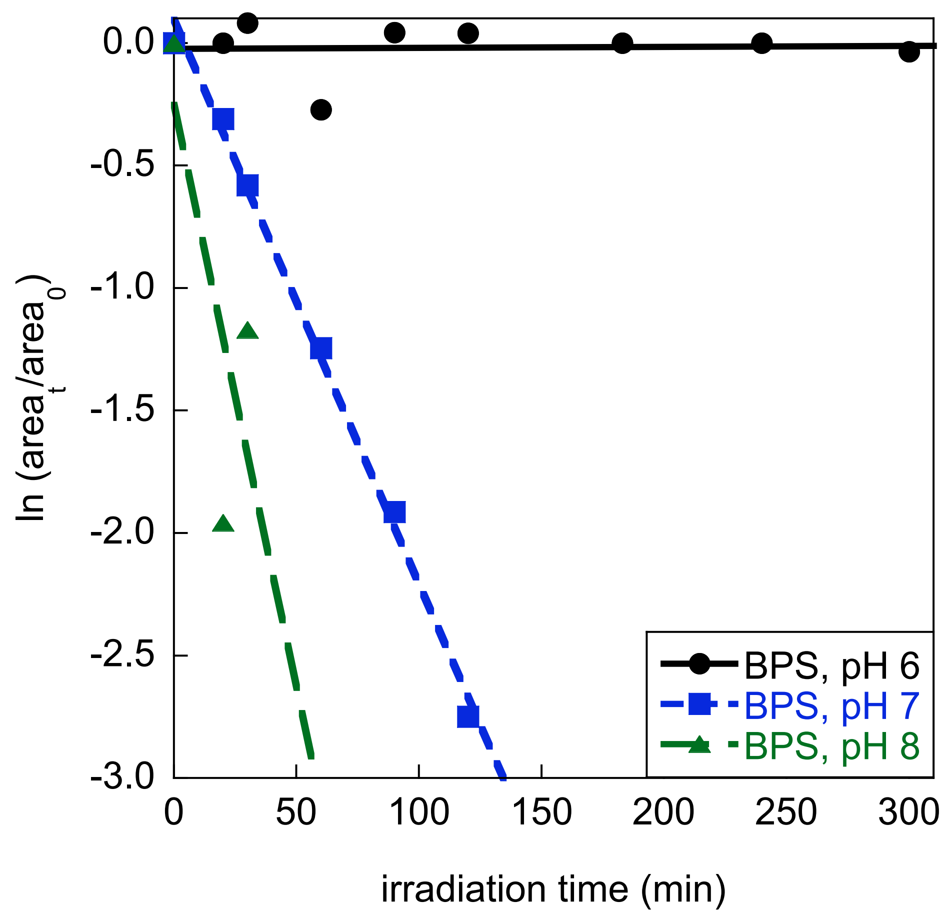
Initial studies of BPs in the research lab



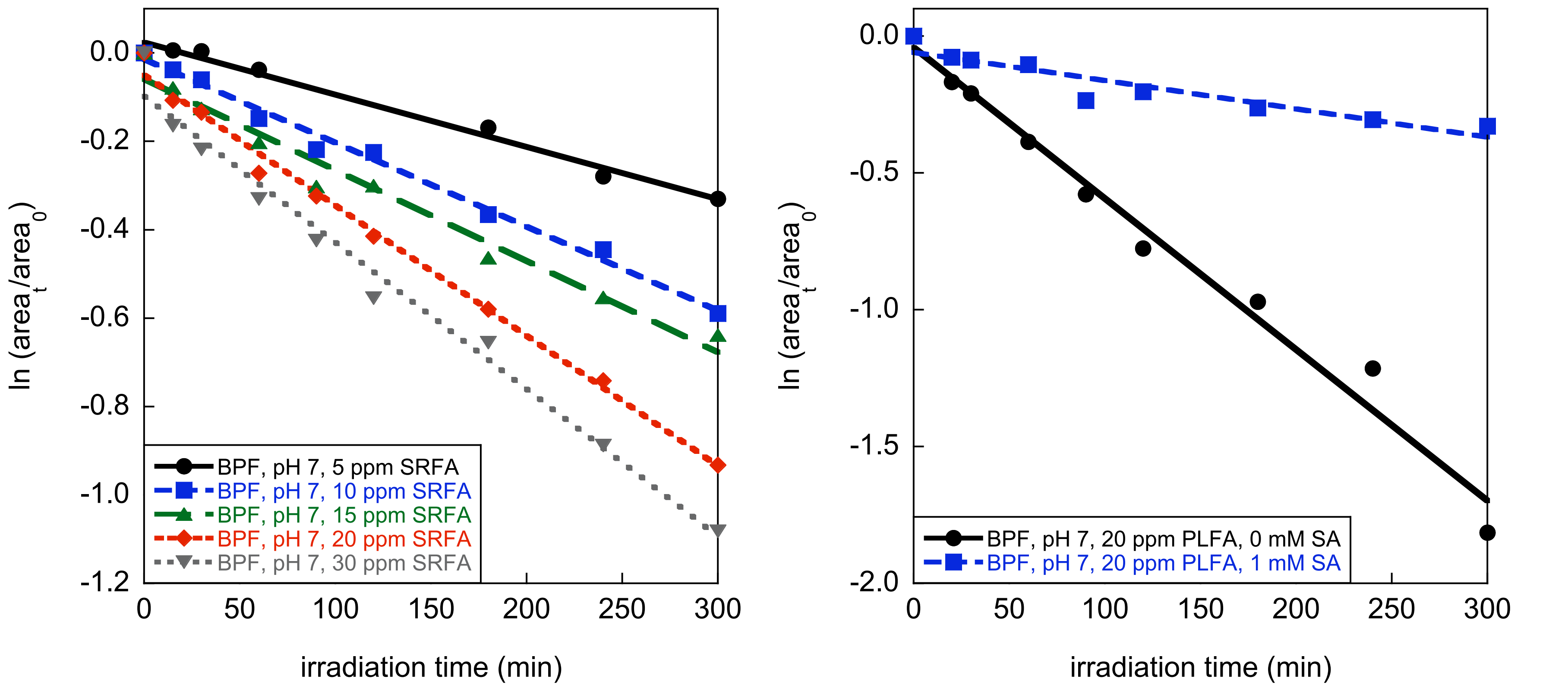


Initial studies of BPs in the research lab: direct





Initial studies of BPs in the research lab: BPF



Insights

from students:

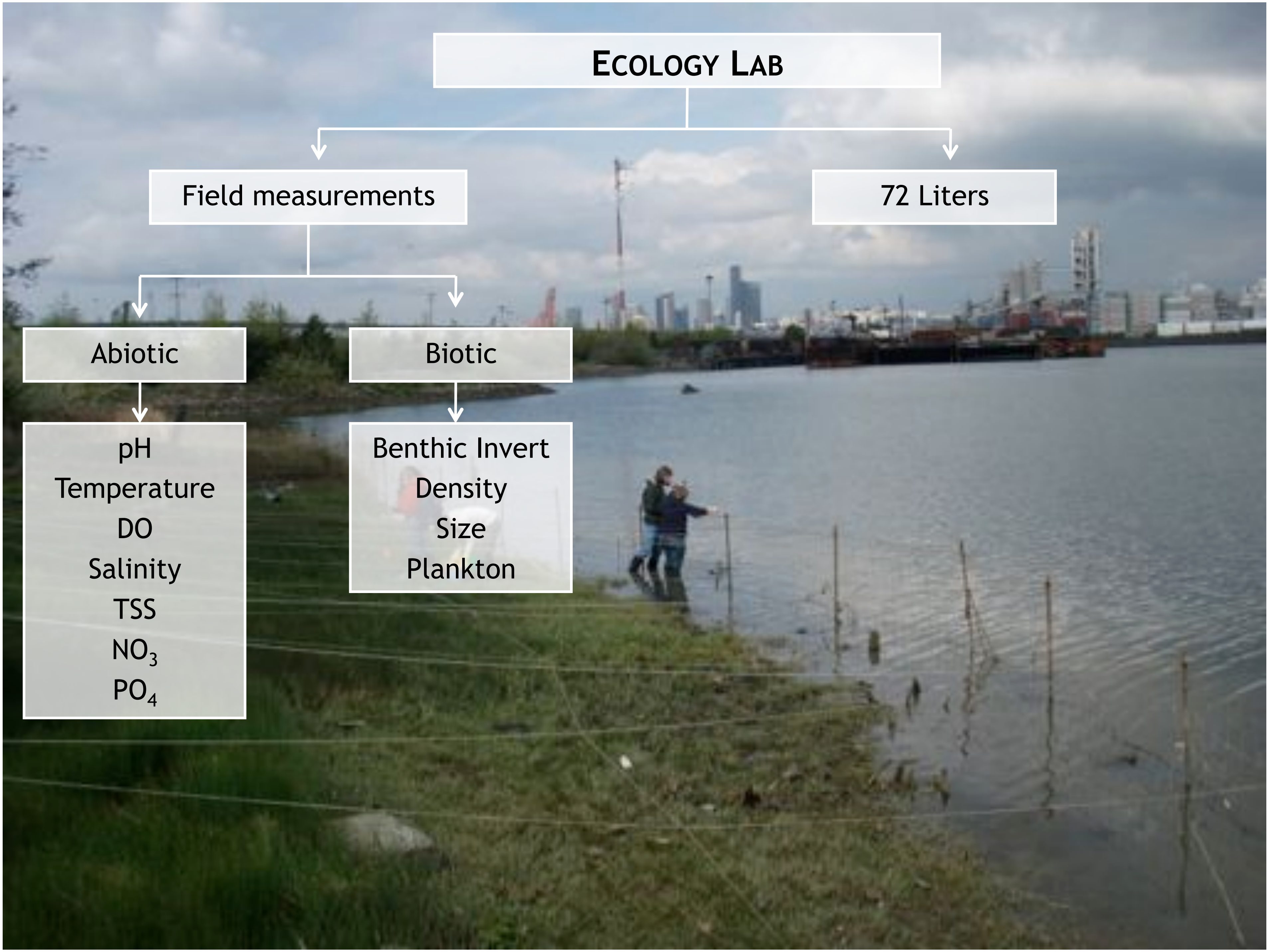
- Trace-level analysis is difficult!
- Instruments can be finicky and experiments do not always work as planned
- "Real world" research is more satisfying but also more difficult than "cookbook" experiments

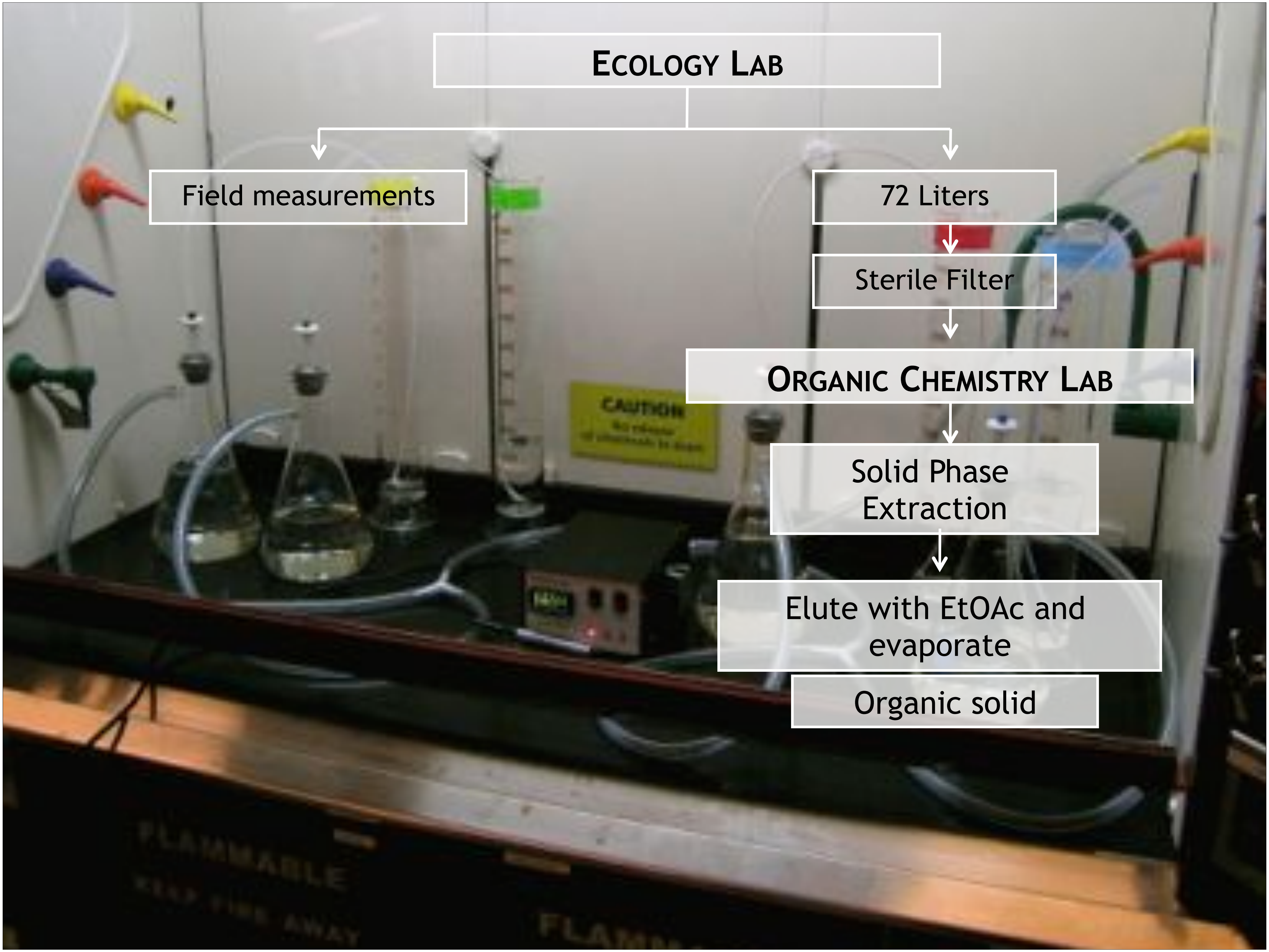
from faculty:

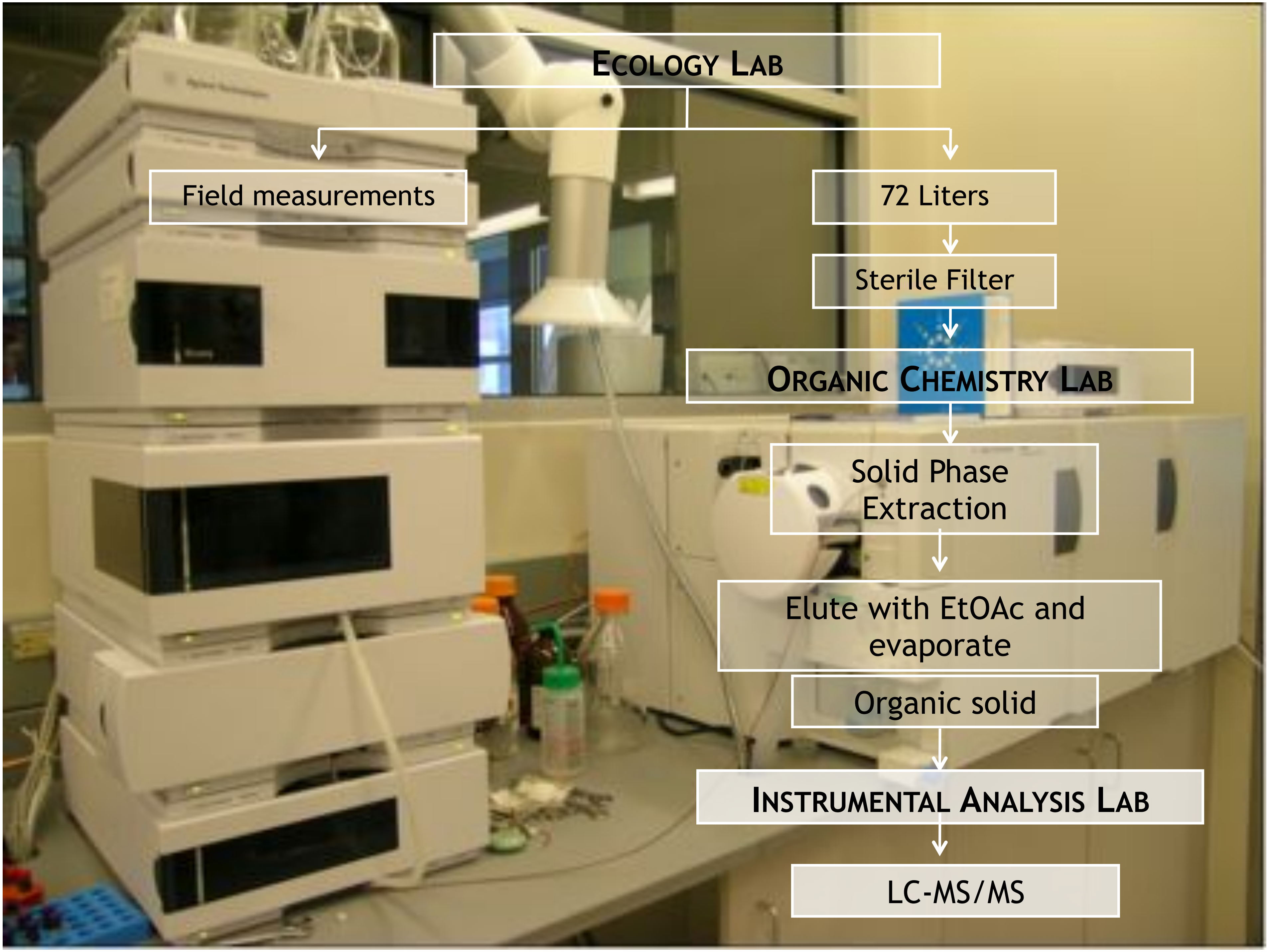
- Students enjoy applying their skills to a good cause
- Students desire more interaction with their ecology colleagues
- Students gain experience using sophisticated equipment
- This course is a lot of work, but it is more enjoyable than the more traditional course had been

Acknowledgements

- Prof. Lindsay Whitlow (SU Biology)
- Prof. PJ Alaimo (SU Chemistry)
- Research students from the Whitlow and Latch groups
  - especially: Lindsay Youngquist, Ann Frost, Chris Whidbey, and John Berude
- the students in the courses (patience and flexibility required)
- Thanh-Hoa Nguyen and Lauren Ryon (teaching assistants; extreme amounts of patience and flexibility required!)
- SU College of Science and Engineering Dean's Office for funding aspects of this project

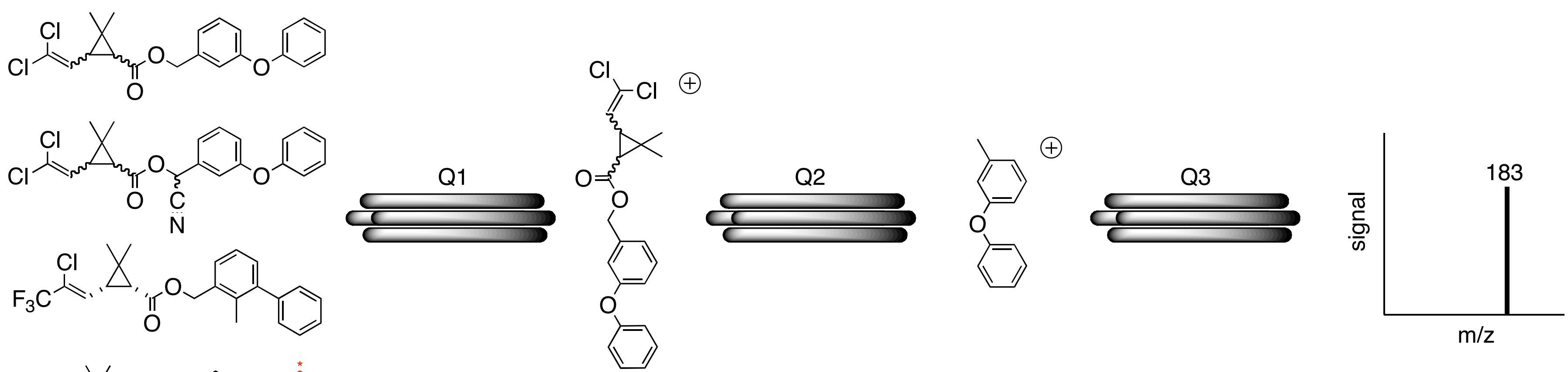

ECOLOGY LAB
Field measurements
72 Liters
Abiotic
Biotic
pH
Temperature
DO
Salinity
TSS
NO_3
PO_4
Benthic Invert
Density
Size
Plankton


ECOLOGY LAB
Field measurements
72 Liters
Sterile Filter
ORGANIC CHEMISTRY LAB
Solid Phase Extraction
Elute with EtOAc and evaporate
Organic solid
CAUTION
FLAMMABLE
KEEP FIRE AWAY



Ecology Lab
Field measurements
72 Liters
Sterile Filter
Organic Chemistry Lab
Solid Phase Extraction
Elute with EtOAc and evaporate
Organic solid
Instrumental Analysis Lab
LC-MS/MS

MS/MS: Sensitive and selective detection



product mixture prior to MS

the first quadrupole acts as a filter, letting ions only with our desired mass-to-charge ratio (m/z) to pass through

at the second quadrupole, the ions that make it past the filter of quadrupole 1 are fragmented by a stream of nitrogen gas

the third quadrupole separates the fragment ions before they reach the detector. The detector records the characteristic mass spectrum