THESIS
The Application of Fourier-Transform Infrared Spectroscopy (FTIR) in Harmful Algal Bloom Characterization

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Overview

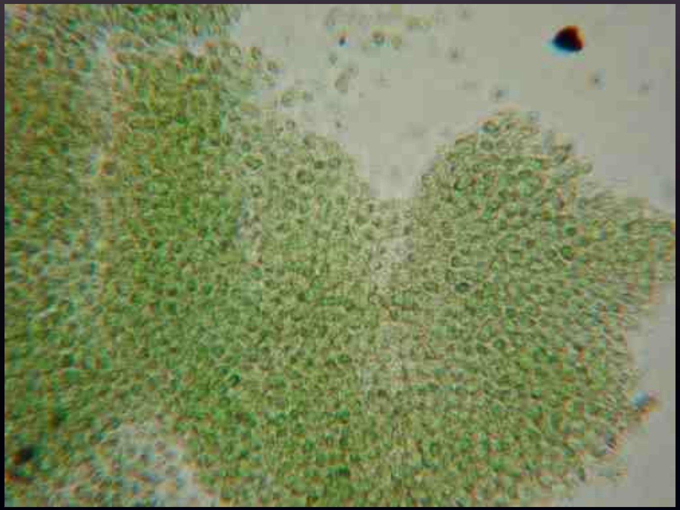
1. Harmful Algal Blooms
   – Environmental and Public Health Impacts

2. Characterization Methods

3. Fourier-Transform Infrared Spectroscopy
   – Practical use in HAB characterization

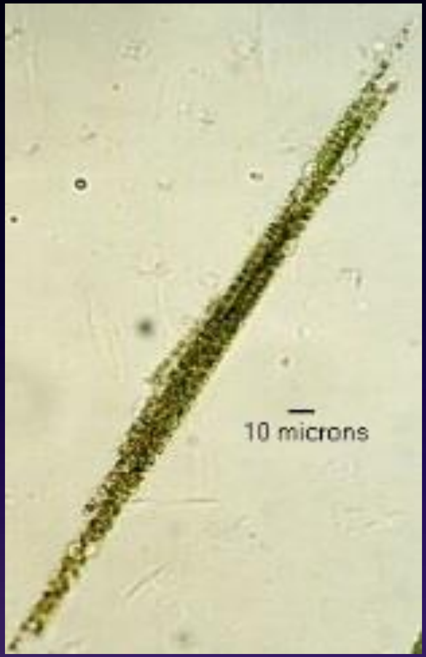
Harmful Algal Blooms (HABs)

- Cyanobacteria (blue-green algae)
  - *Microcystis*
  - *Anabaena*
  - *Aphanizomenon*
  - *Planktothrix*
  - *Cylindrospermopsis*
  - *Oscillatoria*



Microcystis
http://www-cyanosite.bio.purdue.edu/images/lgimages/microcy12.jpg

Anabaena
http://www-cyanosite.bio.purdue.edu/images/lgimages/anabva.jpg



Aphanizomenon
http://www-cyanosite.bio.purdue.edu/images/lgimages/APHANZ3.JPG

Planktothrix
http://www-cyanosite.bio.purdue.edu/images/lgimages/plank2.jpg

Oscillatoria
http://www-cyanosite.bio.purdue.edu/images/lgimages/PRINCEP2.JPG

Harmful Algal Blooms (HABs)

- Surface Blooms
  - Green/Blue-Green in color
  - Paint on water surface
- Benthic Blooms
  - Brown/Orange

http://gallery.usgs.gov/images/06_30_2010/hLc5FSq11Y_06_30_2010/large/Cyanobacteria_22.jpg

- Not limited to standing water bodies:
  - Lakes, Ponds, & Rivers

http://www.michigannow.org/2013/03/27/maddening-algae-on-lake-erie/

Cyanotoxins

Cyanotoxin	Basic Structure	Type	Genera
microcystins	cyclic peptides	hepatotoxin	*Microcystin* *Anabaena* *Oscillatoria* *Planktothris* *Nostoc*
anatoxin-a	alkaloid	neurotoxin	*Anabaena* *Oscillatoria* *Aphanizomenon* *Cylindrospermopsis*
anatoxin-a(s)	guanidinium methyl phosphate ester	neurotoxin	*Anabaena* *Oscillatoria* *Aphanizomenon* *Cylindrospermopsis*
saxitoxin	tricyclic carbamate alkaloids	neurotoxin	*Anabaena* *Aphanizomenon* *Cylindrospermopsis*
cylindrospermopsin	tricyclic alkaloids	cytotoxic	*Cylindrospermopsis* *Aphanizomenon* *Umezaka* *Rhadiopsis*

HABs – Public Health Significance

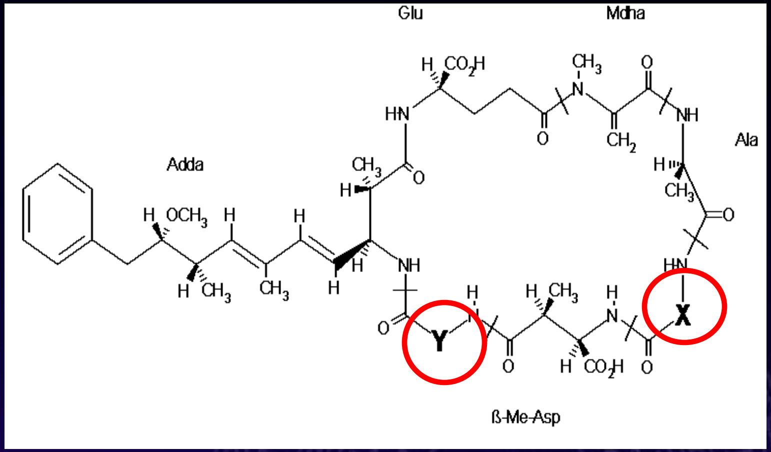
- Human and Animal Illness
- Animal Mortality
  - Companion
  - Livestock
- Environmental Impact
  - Lake/Pond Ecosystems
- Economic Impact
  - Recreational water use
  - Livestock

http://toxics.usgs.gov/photo_gallery/photos/emer_cont/cyanobac/DeadFish_BinderLakeIA_7_l.jpg

Bloom Characterization

Cyanobacteria

Cyanotoxin

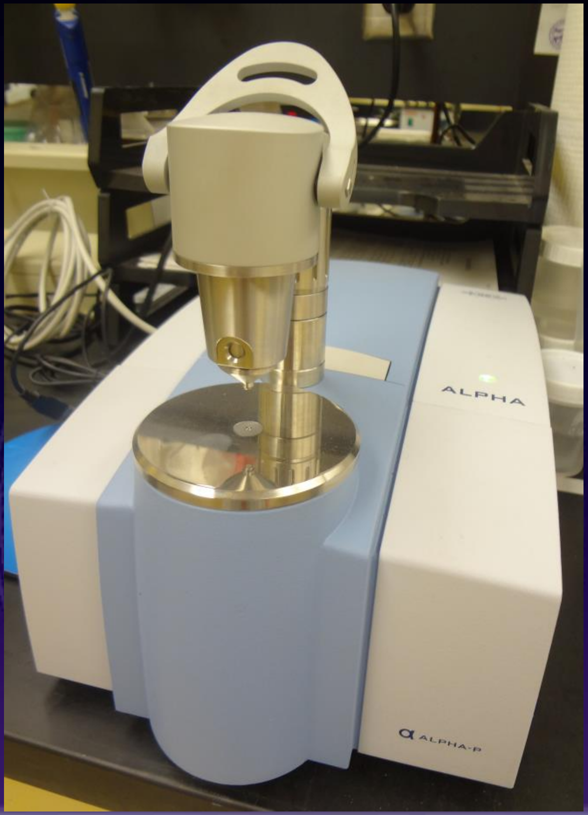


http://www.cyanobacteria-platform.com/cyanotoxins.html

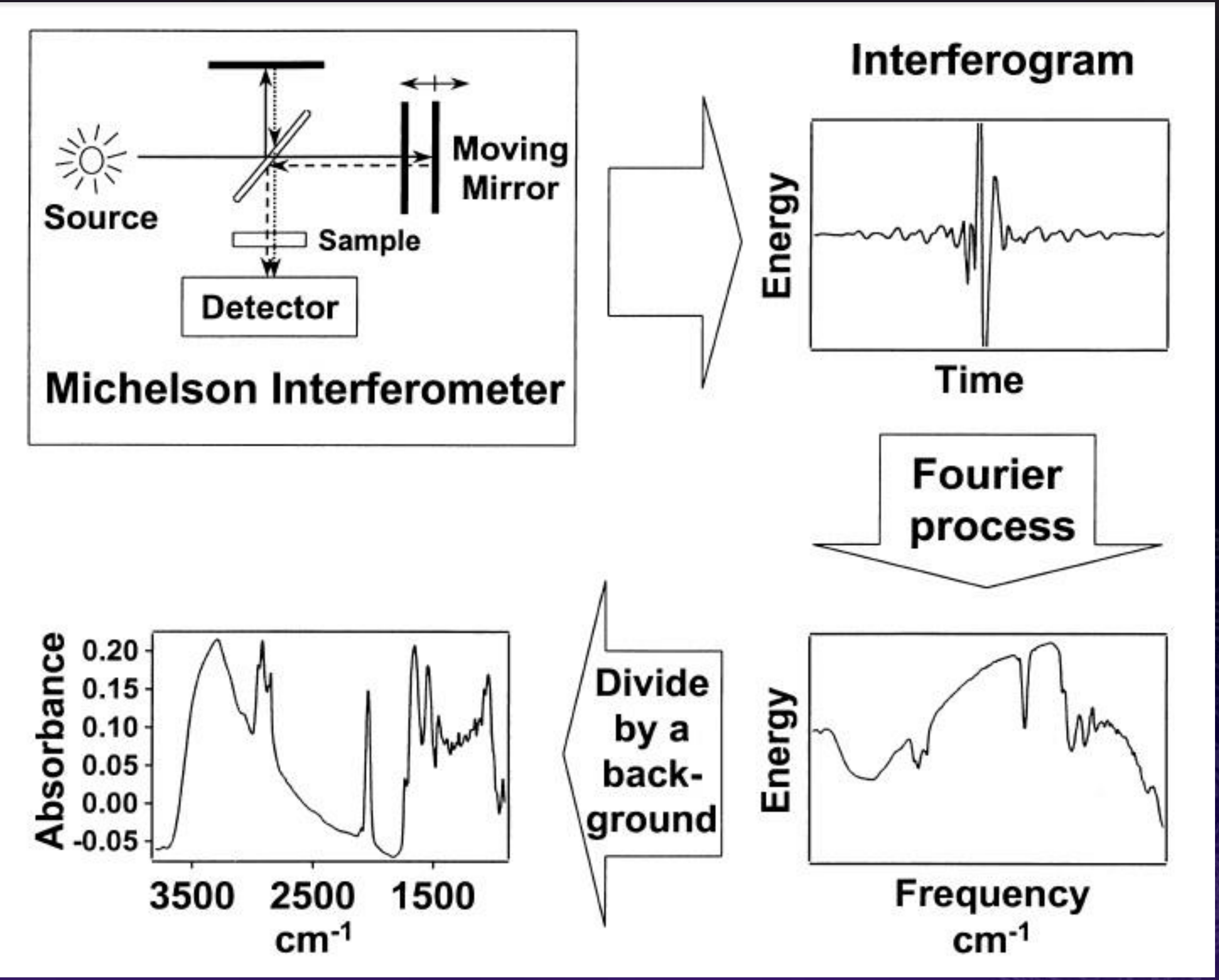
Current Characterization Methods

- Microscopy
- Bioassays
- ELISA
- Protein Phosphatase Inhibition Assay
- PCR
- HPLC
- LC/MS

Using FTIR to Classify Potentially Toxic Cyanobacteria



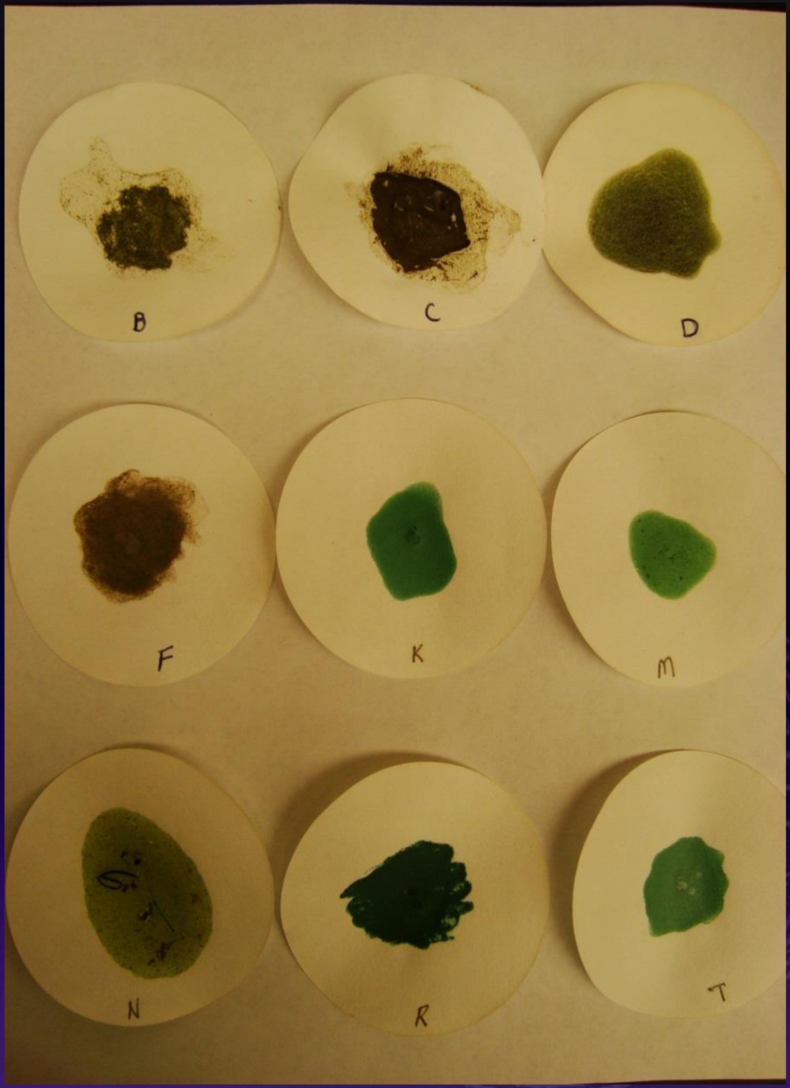
Fourier-Transform Infrared Spectroscopy

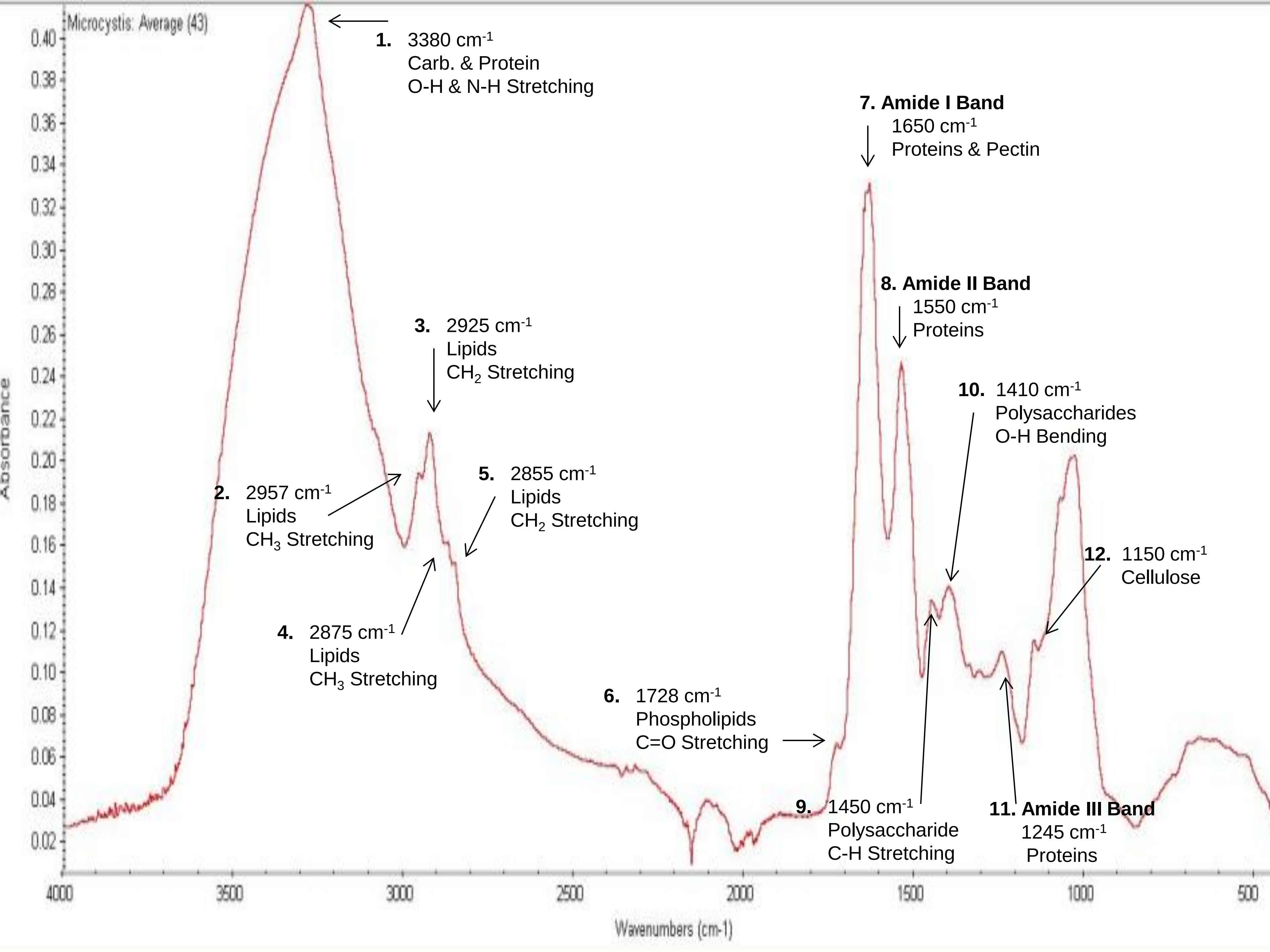


Sacksteder, C. and B. A. Barry (2001). "Fourier Transform Infrared Spectroscopy: A Molecular Approach to and Organismal Question." Journal of Phycology 37: 198.

Methods

- Samples from KSU Vet. Diagnostic Lab
- Dried overnight
- Scanned 3x each
  - Bruker Alpha-P
  - Nicolet 6700




Microcystis: Average (43)
1. 3380 cm-1
Carb. & Protein
O-H & N-H Stretching
2. 2957 cm-1
Lipids
CH3 Stretching
3. 2925 cm-1
Lipids
CH2 Stretching
4. 2875 cm-1
Lipids
CH3 Stretching
5. 2855 cm-1
Lipids
CH2 Stretching
6. 1728 cm-1
Phospholipids
C=O Stretching
7. Amide I Band
1650 cm-1
Proteins & Pectin
8. Amide II Band
1550 cm-1
Proteins
9. 1450 cm-1
Polysaccharide
C-H Stretching
10. 1410 cm-1
Polysaccharides
O-H Bending
11. Amide III Band
1245 cm-1
Proteins
12. 1150 cm-1
Cellulose
Absorbance
Wavenumbers (cm-1)
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
4000
3500
3000
2500
2000
1500
1000
500

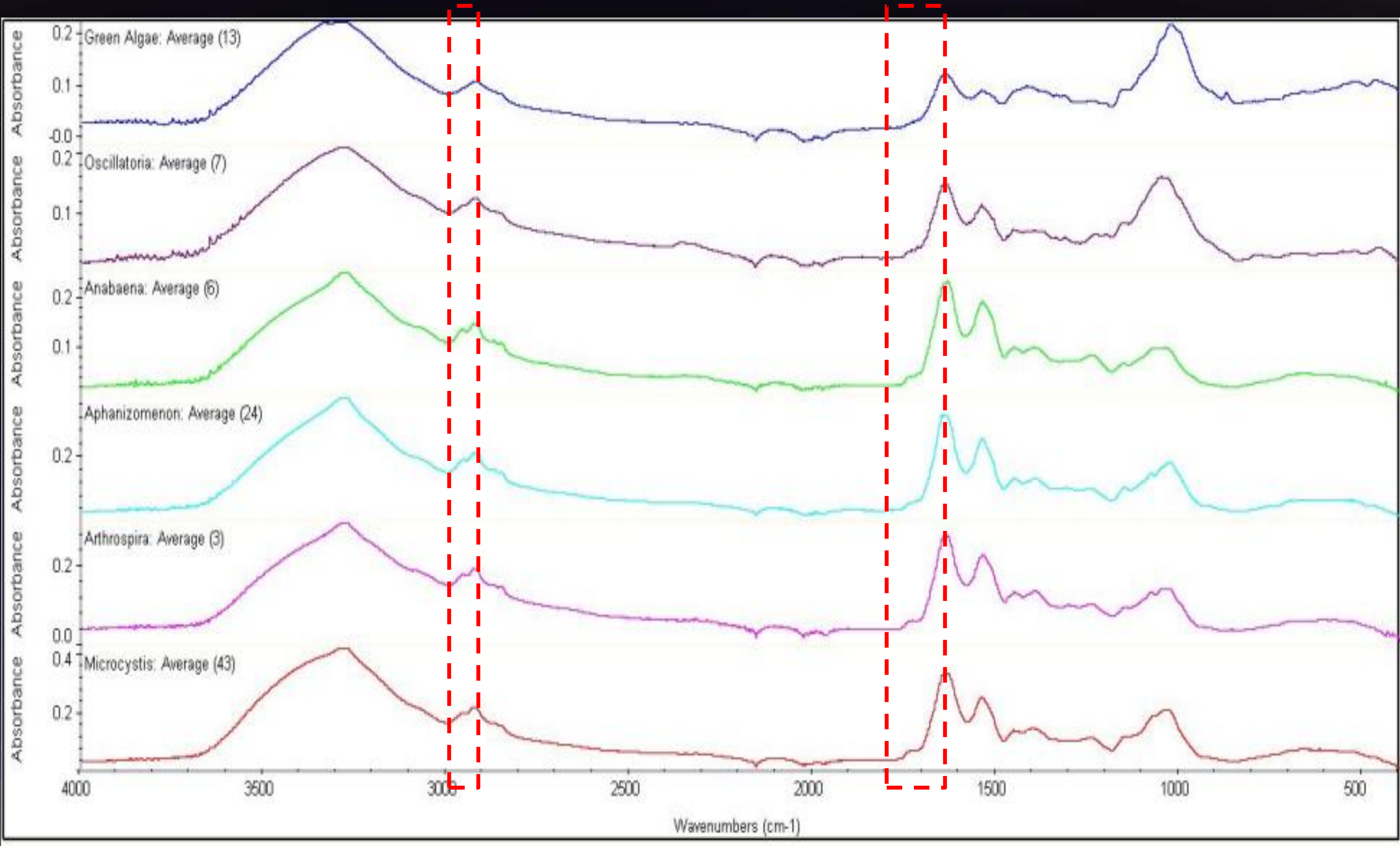
Methods

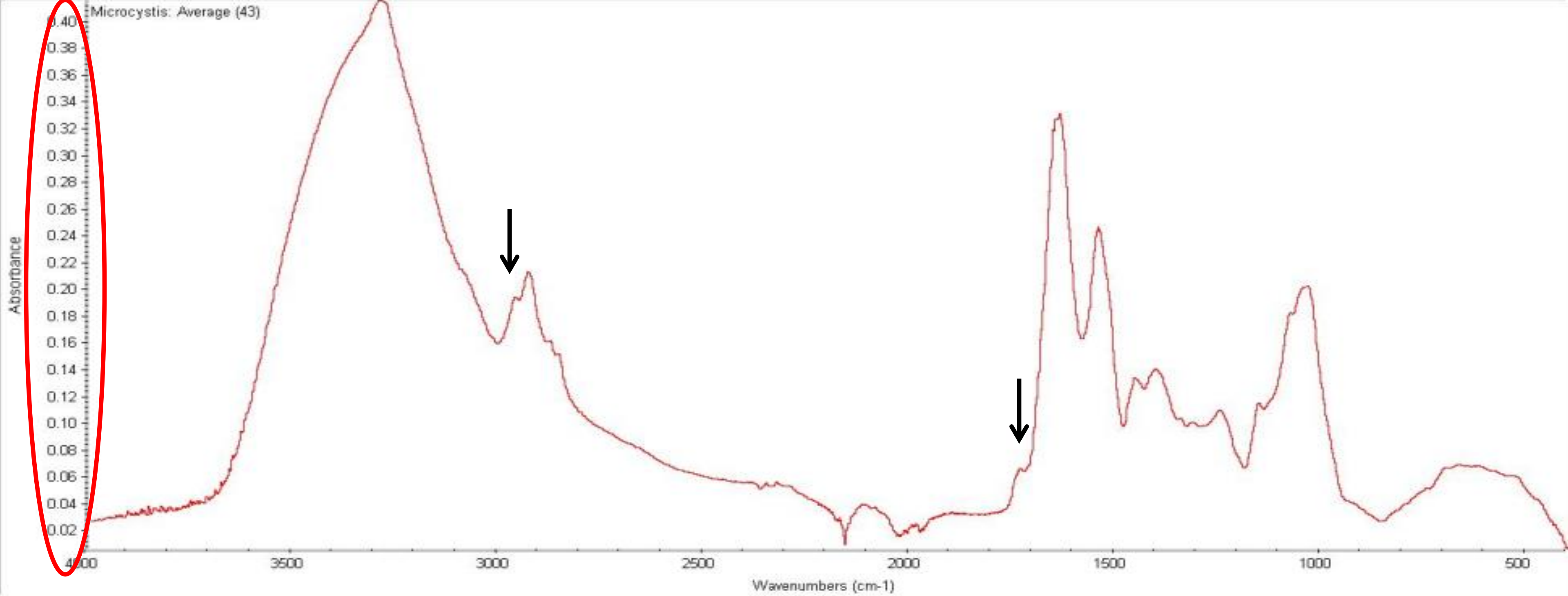
- Data Tables
  - OMNIC Software (Nicolet 6700)
  - OPUS Software (Bruker Alpha-P)
  - Normalized to Amide I Band (Band 7)

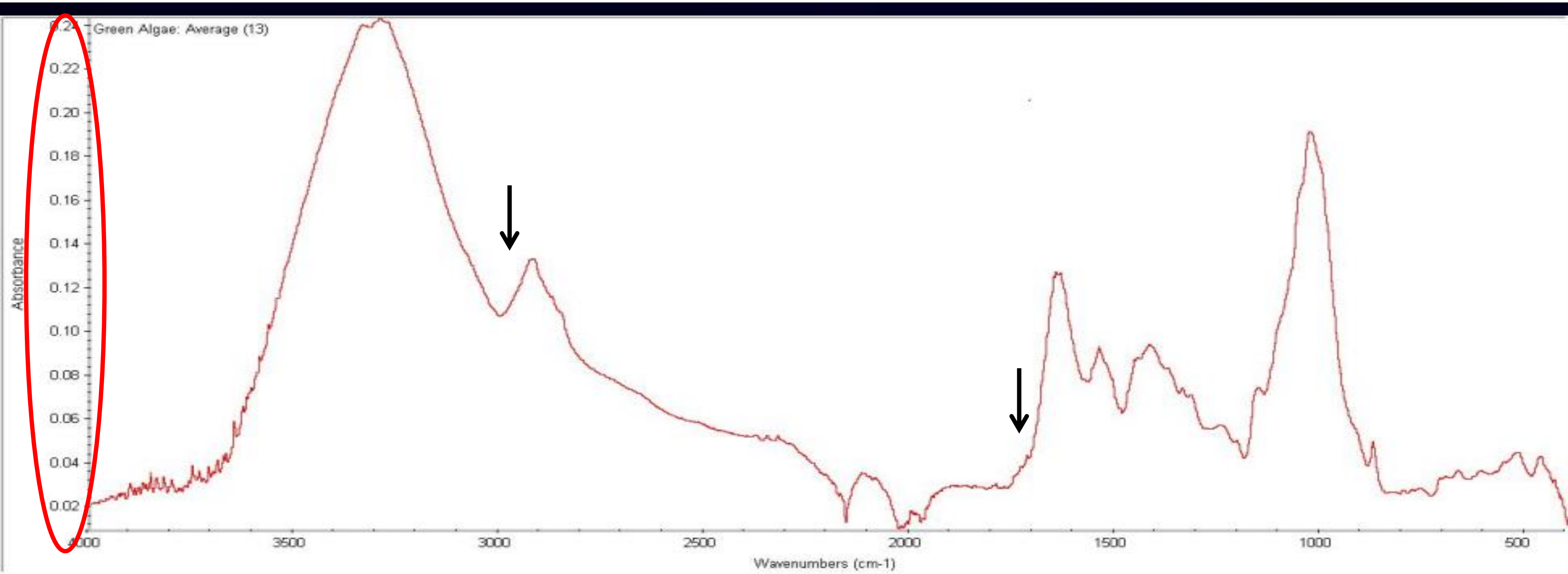
- Data Analysis

1. Spectral Observations
2. Default Algorithm Screening
3. Principle Component Cluster Analysis
4. Dendrograms

1. Spectral Observations




Microcystis: Average (43)
Absorbance
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Green Algae: Average (13)
Absorbance
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

2. Default Software Algorithms

Single-Genera Samples:

- OPUS
  - 29 of 31 samples correctly classified as cyanobacteria or not cyanobacteria*
    - 0.9355
- OMNIC
  - 28 of 31 samples correctly classified
    - 0.9032

All Samples: **(includes mixed samples)**

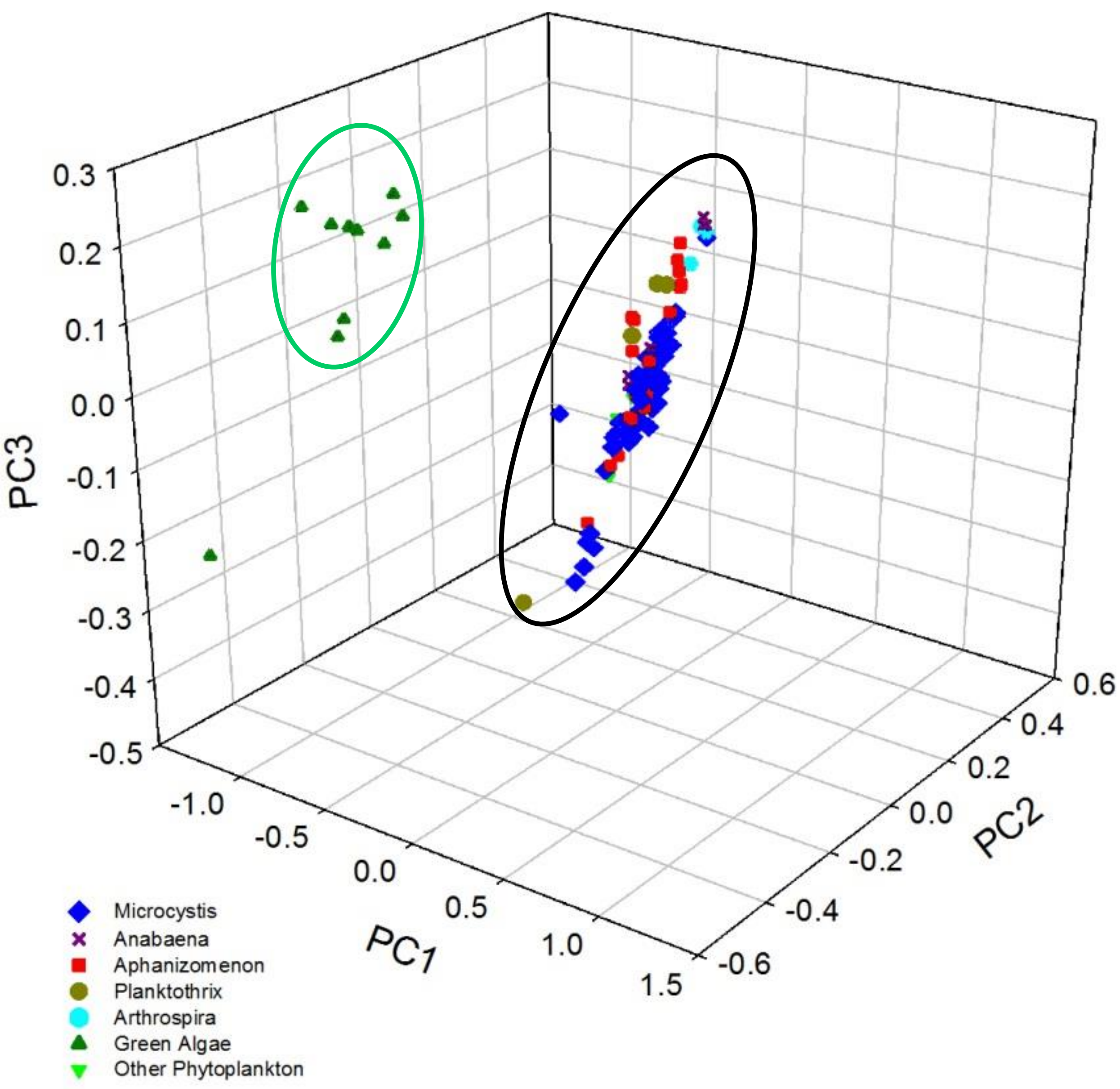
- OPUS
  - 36 of 43 samples correctly classified
    - 0.8372
- OMNIC
  - 35 of 43 samples correctly classified
    - 0.8140

*Classification of specific cyanobacterial genera was more variable and less successful

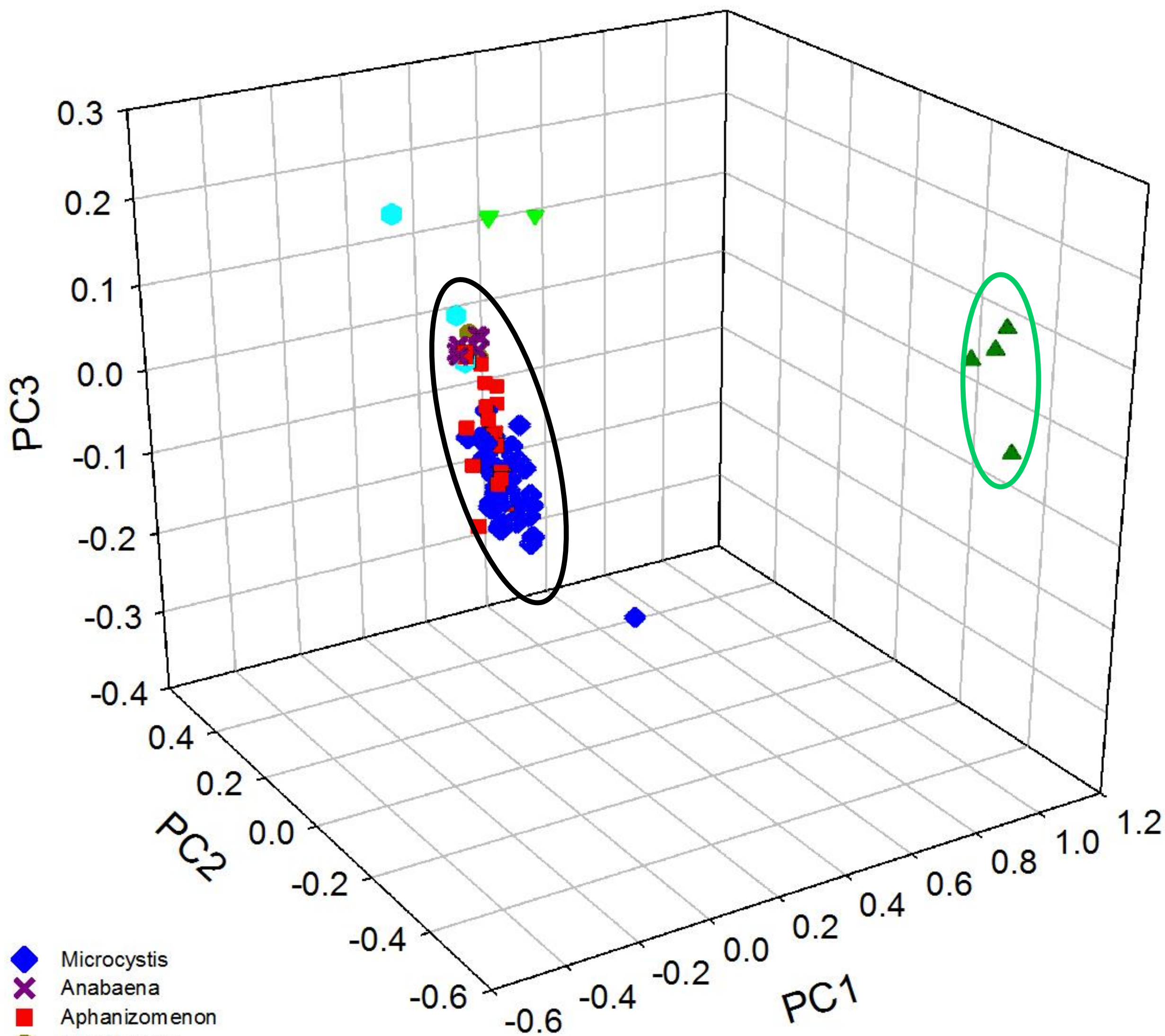
3. Principle Component Cluster Analysis

- MatLab 7.7.0
- Single-Genera Samples – Normalized Peak 1-12 Data
- Poor quality samples excluded based on raw Amide I band height
  - OPUS: ≤ 0.060
  - OMNIC ≤ 0.100
- **113 OMNIC Spectra**
- **91 OPUS Spectra**

OMNIC Principle Component Cluster Analysis

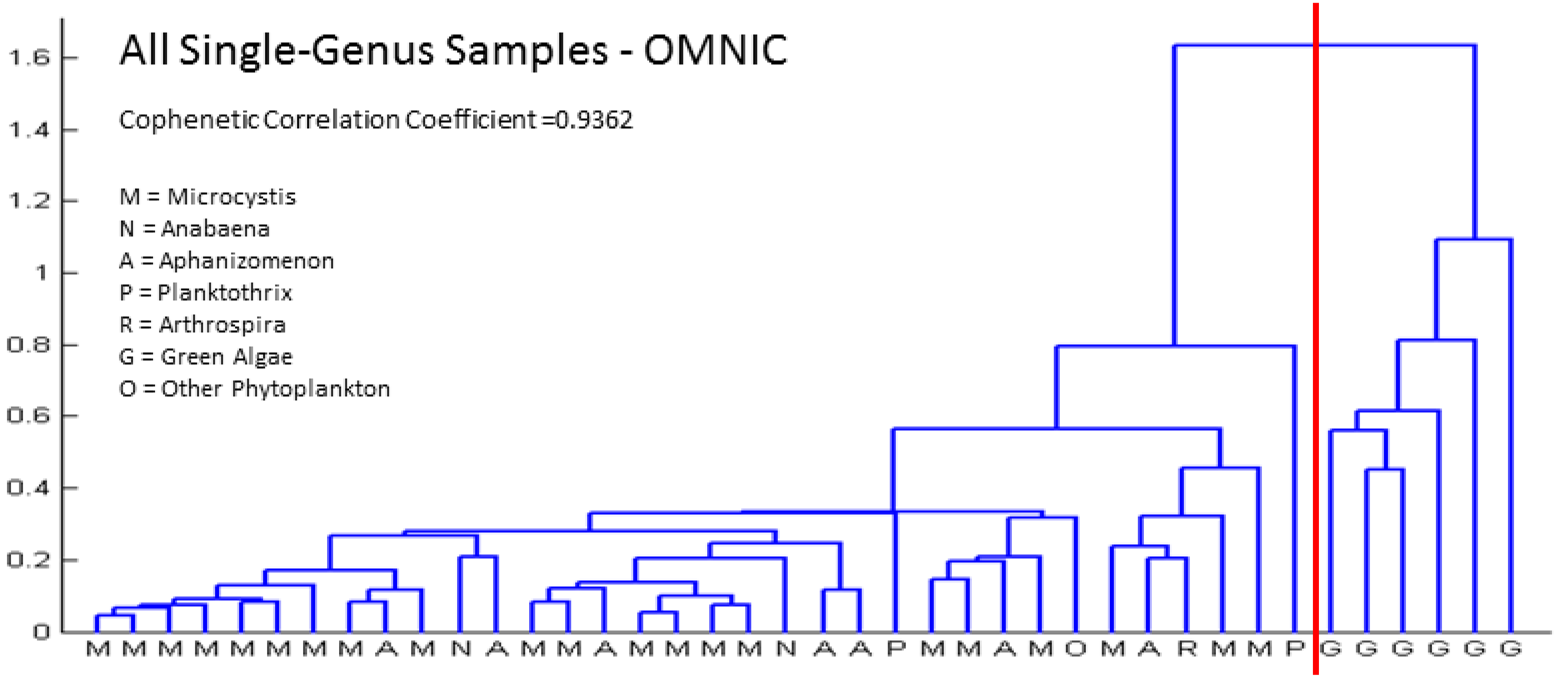


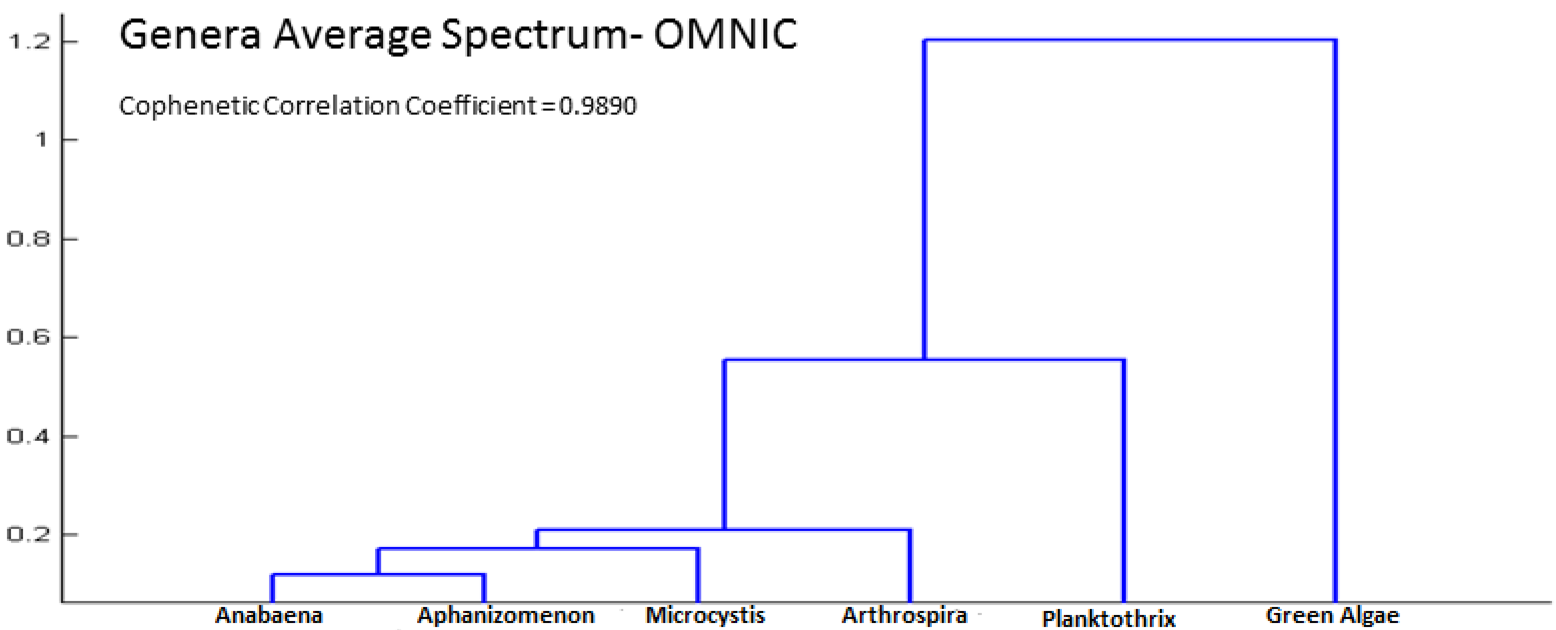
OPUS Principle Component Cluster Analysis

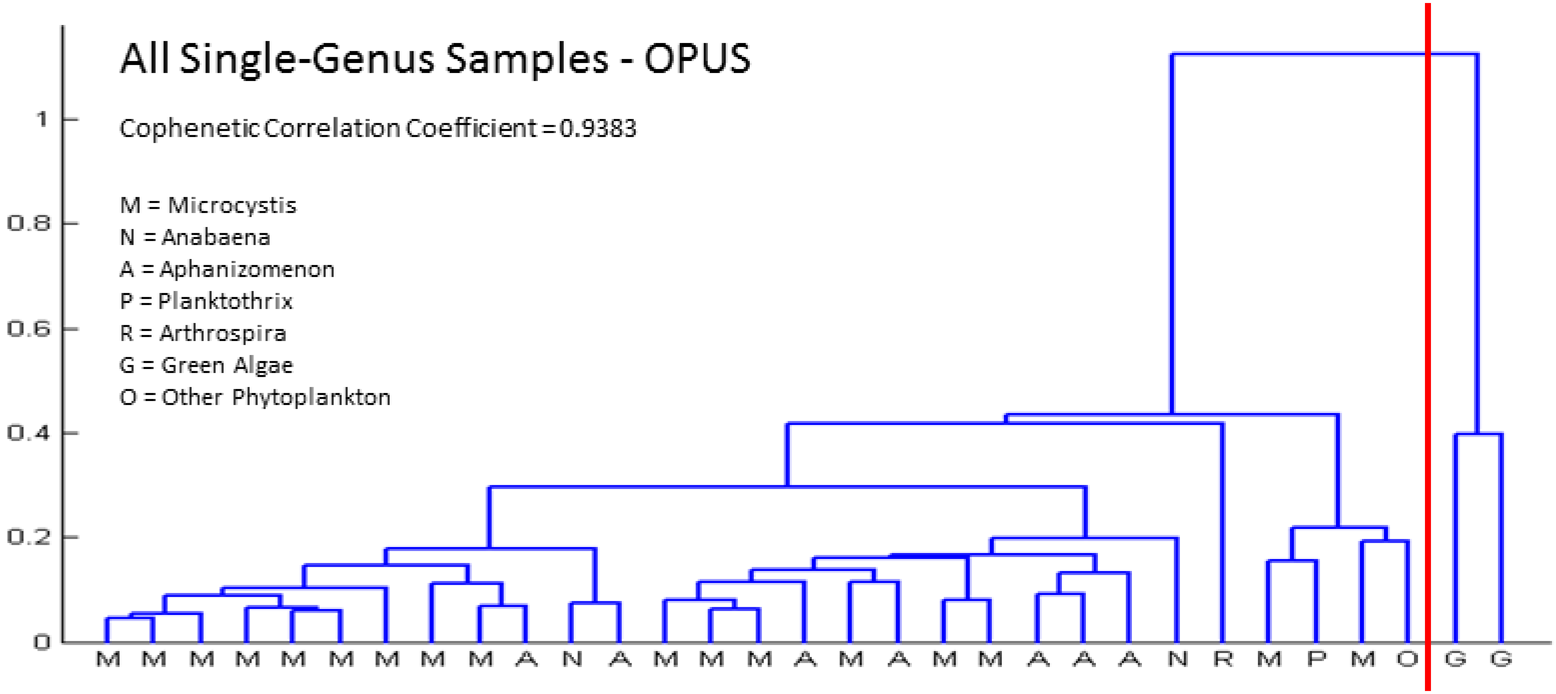


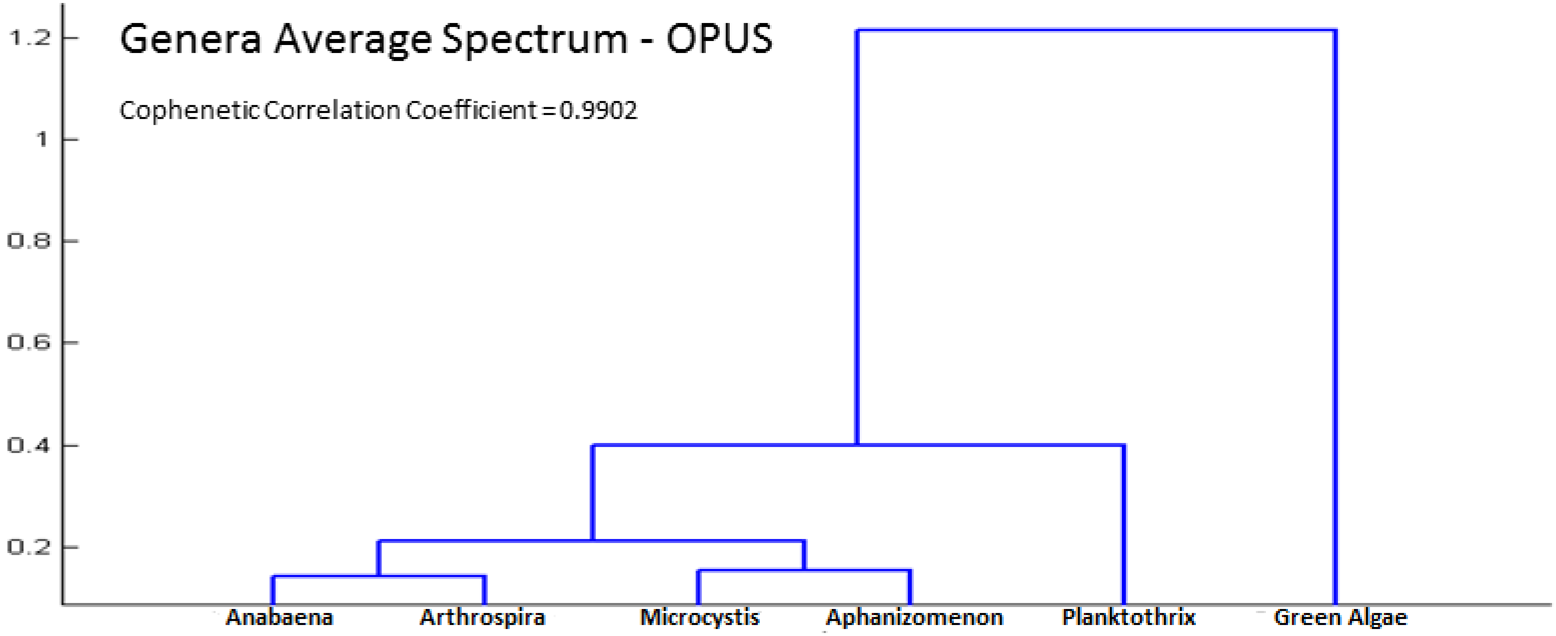
4. Dendrograms

- MatLab 7.7.0
- Calculated using Average Euclidian Distance:
  - Average Spectra
  - All Samples
    - 1 Spectrum/Sample


All Single-Genus Samples - OMNIC
Cophenetic Correlation Coefficient =0.9362
M = Microcystis
N = Anabaena
A = Aphanizomenon
P = Planktothrix
R = Arthrospira
G = Green Algae
O = Other Phytoplankton
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
M M M M M M M M A M N A M M A M M M M N A A P M M A M O M A R M M P G G G G G G


Genera Average Spectrum- OMNIC
Cophenetic Correlation Coefficient =0.9890
1.2
1
0.8
0.6
0.4
0.2
Anabaena
Aphanizomenon
Microcystis
Arthrospira
Planktothrix
Green Algae


All Single-Genus Samples - OPUS
Cophenetic Correlation Coefficient = 0.9383
M = Microcystis
N = Anabaena
A = Aphanizomenon
P = Planktothrix
R = Arthrospira
G = Green Algae
O = Other Phytoplankton
1
0.8
0.6
0.4
0.2
0
M M M M M M M M M A N A M M M A M A M M A A A N R M P M O G G


Genera Average Spectrum - OPUS
Cophenetic Correlation Coefficient = 0.9902
1.2
1
0.8
0.6
0.4
0.2
Anabaena
Arthrospira
Microcystis
Aphanizomenon
Planktothrix
Green Algae

Conclusions

- The success of FTIR in classifying cyanobacteria from environmental samples demonstrates its usefulness in HAB characterization.
  - Sample Quality
  - In conjunction with toxin detection methods

1. Screening Tool
2. Diagnostics

Future Research

- Sample quality limitations
- Specific Genera
- Using dried samples for toxin identification

MPH FIELD EXPERIENCE: KDHE

Spatial and Temporal Analysis of Kansas Childhood Blood Lead Levels : 2006-2011

Field Experience

- Kansas Department of Environmental Health
  - Bureau of Environmental Health (BEH)
  - Environmental Public Health Tracking (EPHT)
    - Henri Menager

Purpose

- CDC recommends a targeted screening program to prevent childhood lead exposures
  - State Funding Cut in Aug. 2012
  - Systematic Tracking of Elevated Blood-Lead Levels and Remediation (STELLAR)

- Using data from the STELLAR database, identify spatial and demographic “high risk” target zones in the state of Kansas for the Healthy Homes and Lead Hazard Prevention Program (HHLHPP)

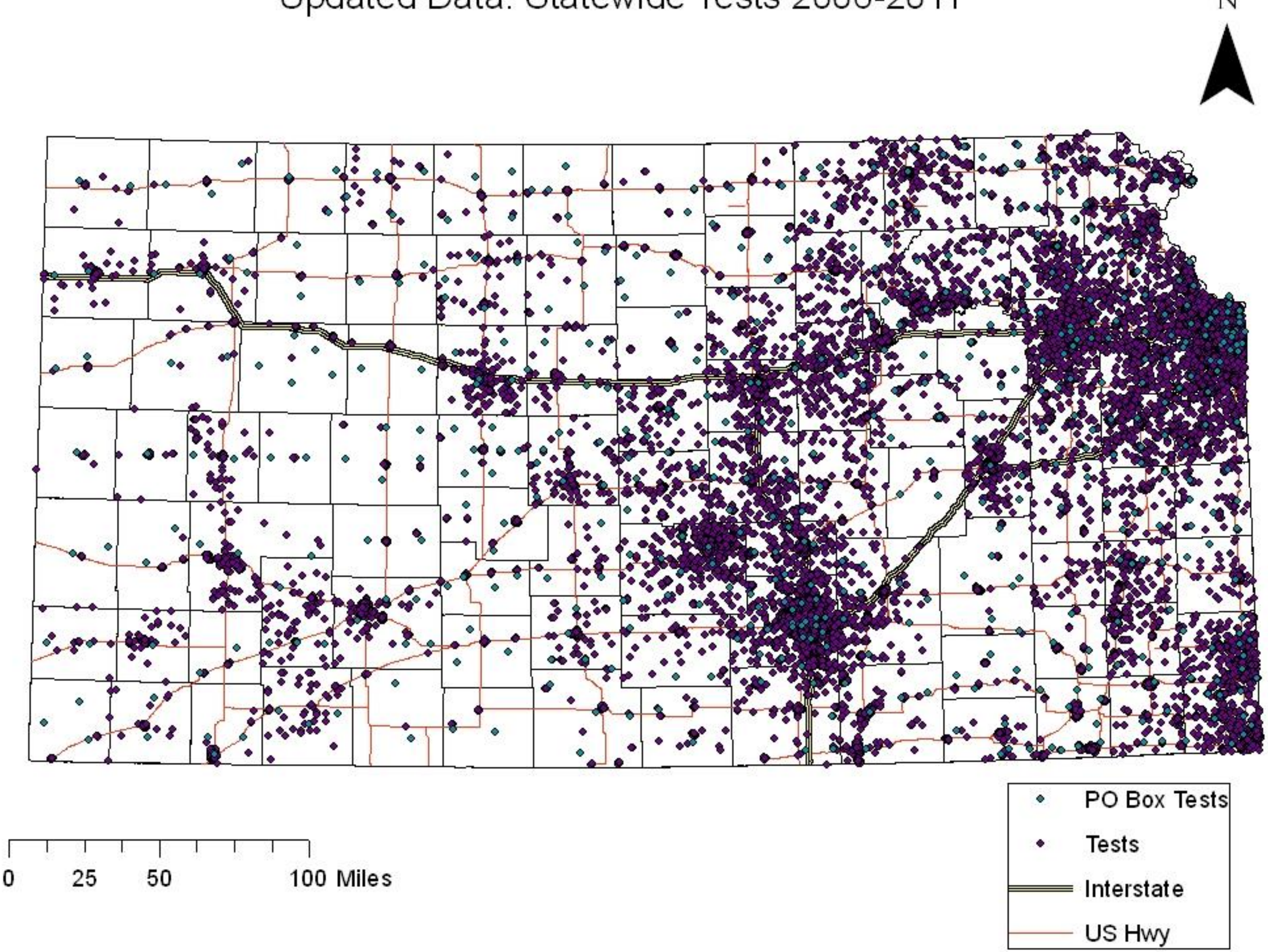
Methods

- Data Acquisition:
  - STELLAR
    - Jan. 1, 2006 – Dec. 31, 2011
    - 0-72 months old
    - 219,090 blood lead tests
  - Address entry/correction
    - 171,122 valid addresses (78.1%)
    - 8,804 addresses corrected
    - 179,926 total addresses used in spatial analysis (82.1%)
      - 17.9% with no usable address information

Methods

- ArcMap:
  - Data Layers:
    - Census Tract Demographics Data
      - population data by race and age
      - household income
      - percentage individuals below the poverty level
  - Blood Lead Tests
  - Cases of Lead Poisoning

Updated Data: Statewide Tests 2006-2011



Childhood Blood Lead Poisoning

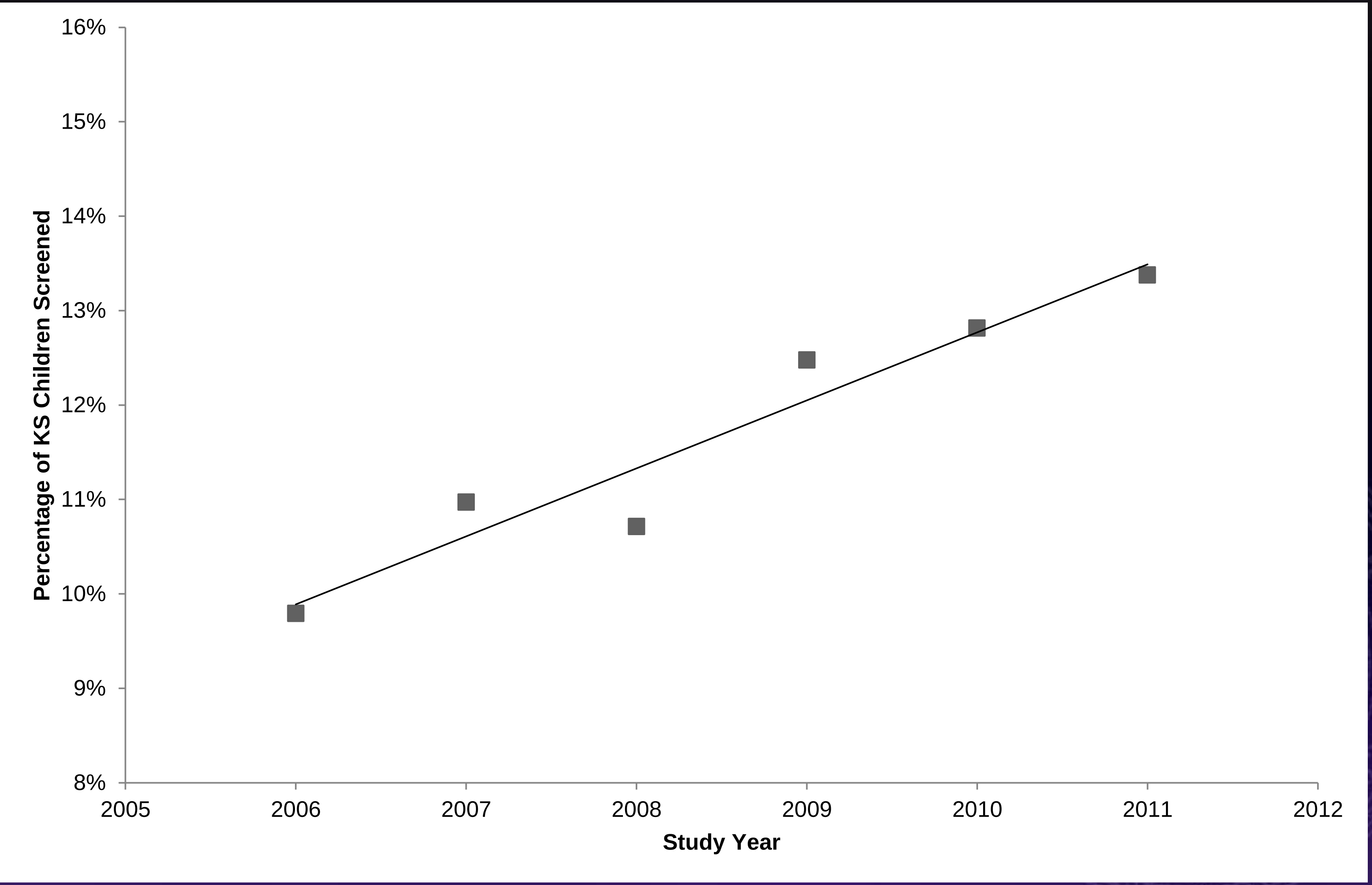
- Case (Confirmed Elevated Blood Lead)
  - ≥ 10μg/dL lead present in a venous blood draw, or
  - ≥ 10μg/dL lead present in TWO capillary blood draws
    - Must be within 90 days of each other to be a case

Observations

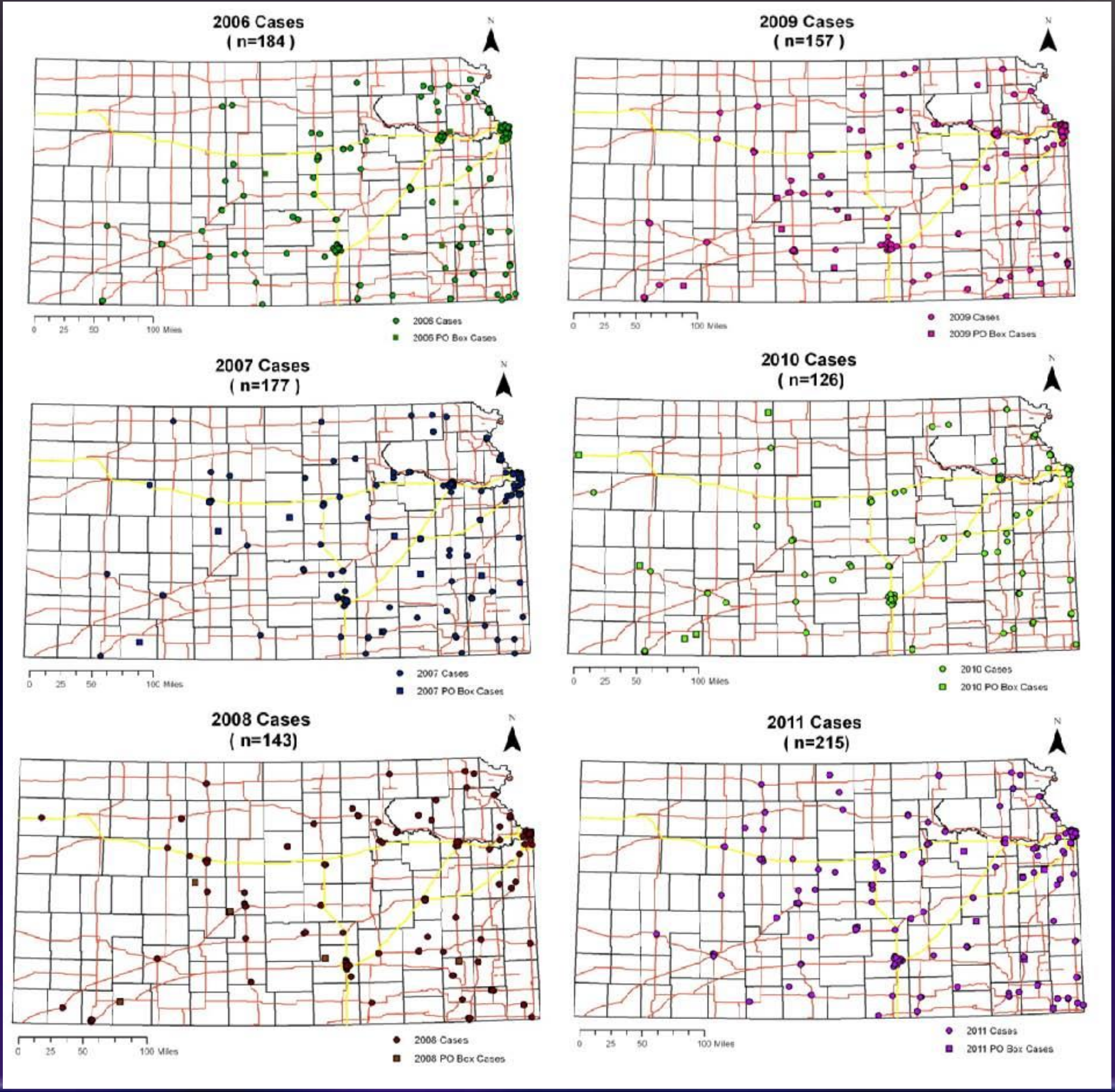
1. Screening Rates per Year*
2. Case Distribution by Year
3. Cases in High Poverty Regions
4. Demographic Target Zones

*Rates based on county birth cohorts to calculate all children 0-72 months old during the calendar year.

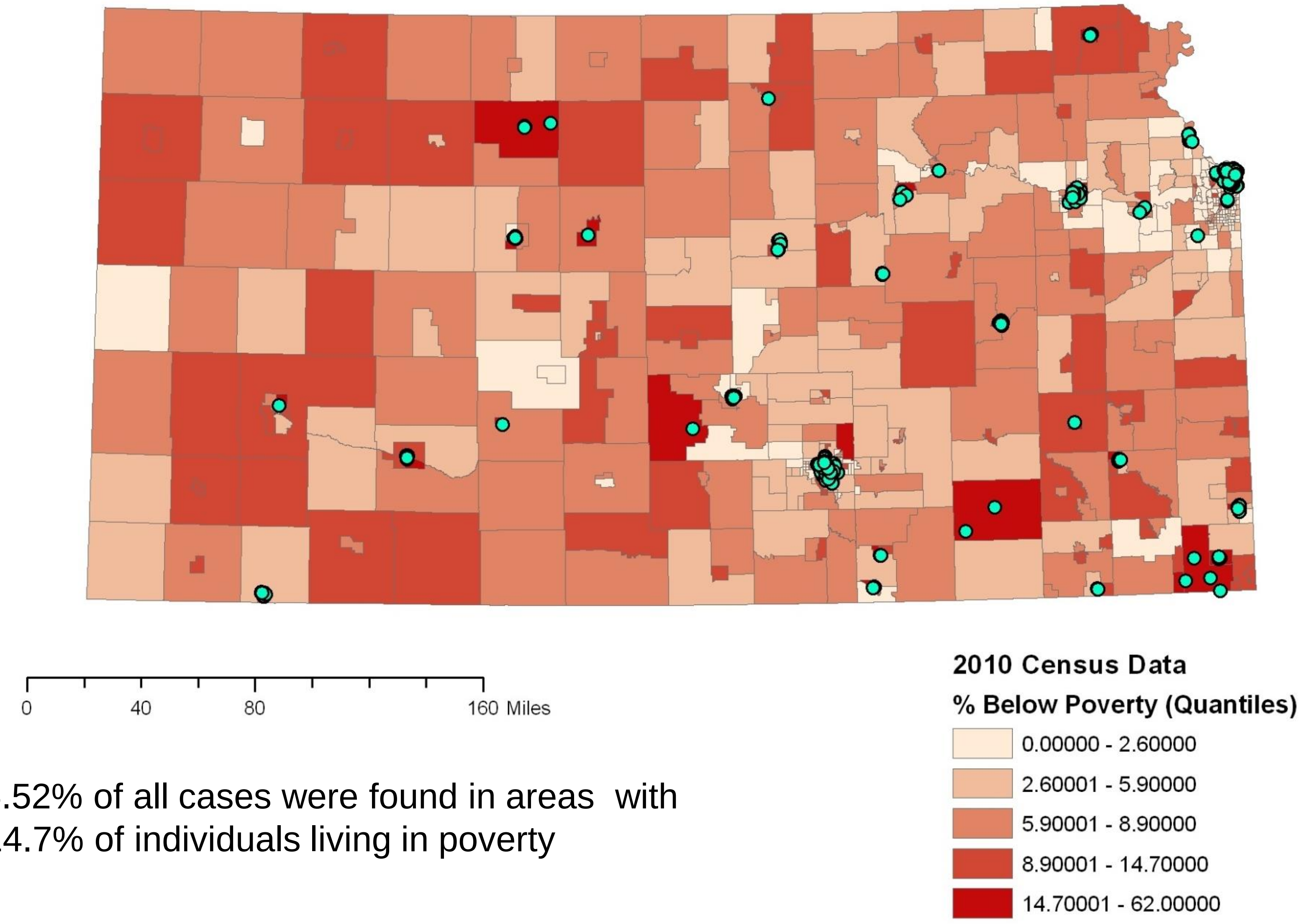
1. Statewide Screening Rates/Year



2. Case Distribution by Year



3. Cases found in High Poverty Regions (384)



16.52% of all cases were found in areas with >14.7% of individuals living in poverty

4.Race & Ethnicity

All Tests	2006	2007	2008	2009	2010	2011	2006-2011 Total
White	7835	6950	2967	1570	1125	845	21292
Black	1591	1447	693	400	239	178	4548
Asian/Pacific Islander	59	85	27	20	16	10	217
Native American	48	37	10	6	3	0	104
Multiracial	4	3	0	0	1	0	8
Unknown	22429	26043	32898	36529	37099	37923	192921
Hispanic	1556	1681	853	399	375	253	5117
Non-Hispanic	2734	3210	1417	705	421	256	8743
Other	2	0	1	0	0	0	3
Unknown	27674	29374	34324	37421	37687	38447	204927
State Total	31966	34565	36595	38525	38483	38956	219090

% of All Tests	2006	2007	2008	2009	2010	2011	2006-2011 Total
White	24.510%	20.107%	8.108%	4.075%	2.923%	2.169%	9.718%
Black	4.977%	4.186%	1.894%	1.038%	0.621%	0.457%	2.076%
Asian/Pacific Islander	0.185%	0.246%	0.074%	0.052%	0.042%	0.026%	0.099%
Native American	0.150%	0.107%	0.027%	0.016%	0.008%	0.000%	0.047%
Multiracial	0.013%	0.009%	0.000%	0.000%	0.003%	0.000%	0.004%
Unknown	70.165%	75.345%	89.898%	94.819%	96.404%	97.348%	88.056%
Hispanic	4.868%	4.863%	2.331%	1.036%	0.974%	0.649%	2.336%
Non-Hispanic	8.553%	9.287%	3.872%	1.830%	1.094%	0.657%	3.991%
Other	0.006%	0.000%	0.003%	0.000%	0.000%	0.000%	0.001%
Unknown	86.573%	84.982%	93.794%	97.134%	97.932%	98.693%	93.536%

Limitations

- Spatial and Demographic Data
- Lack of tools and data to correlate target zones limit the value of the spatial analysis.
  - Spatial analysis tools in ArcMap
  - Accurate Housing Age Data*
  - Lead Industries
  - Medicaid Data*

Conclusions

- Quality data is necessary to represent the real-world implications of environmental lead exposure, and crucial to identifying potential target zones for prevention measures.
- Interventions at the physician, laboratory, and data entry level should be made to explain the importance of this data and its necessity for targeted analyses.

Acknowledgements and Program Influences

Individuals

- Dr. Deon van der Merwe
- Dr. Thu Annelise Nguyen
- Dr. Shawn Hutchinson
- Henri Menager

- Lori Blevins
- Ali Mahdi

- Dr. Justin Kastner

Coursework/Organizations

- Toxicology Coursework
- Geographic Information Systems
- Epidemiology

- Frontier Program

Thank You

www.newswise.com/images/uploads/2006/08/23/fullsize/microcystis_shake.jpg;