How can things so small cause such big problems?

• What do you say if a customer asks:
  – "My water smells, is it safe to drink?"
  – "Why should I drink tap water if it smells like dirt?"
  – "If you can’t make it smell and taste good why should I pay for it?"

• What do you say if a WTP operator asks:
  – "What do I do to control in-plant algae growth that causes short circuiting and short filter run times now that you convinced me that our previous practice of prechlorinating may kill people?"

• What do you say when a utility manager asks:
  – "What is the best way to monitor and prevent algae related problems?"
All those questions have been asked recently in Central Arizona

Presentation Outline

• Methods
• Algae Metabolites
  – Bulk organic matter
  – Taste and odors
  – Cyanotoxins
• Ongoing algae activites
  – Genetic fingerprinting of culprit algae
  – Managing nuisance algae
• Conclusions
Methods

• DOC & DON: Shimadzu TOC-V
• DIN: Ion chromatography & phenate method
• T&O: SPME with GC/MS
• Algal toxins:
  – ELISA
  – Protein Phosphatase Inhibition Assay (PPIA)
  – Anatoxin-a/Saxatoxin – HPLC after fluorescent derivatization
  – Cylindrospermopsin – HPLC using a photodiode array detector
• PCR for 16s-RNA genetic fingerprinting

Bulk Organic Matter Production by Algae
Algae release extracellular material (bulk DOC)

DOC production & DBP formation potential with DISSOLVE metabolites from Scenedesmus quadricauda

THM formation potential: 118 µg CHCl₃/mgDOC
Many Algae Metabolites Contain Dissolved Organic Nitrogen

![Graph showing DON (mg/L) over time for different lakes.]

Algae Influence Watersheds Produce NOM (mg/L)

- Laboratory results showed that only 55 to 70% of algae-produced DOC were removable by biodegradation.
- Long-term reservoir storage accumulates refractory algal DOC causing a net gain (~10%) in DOC budgets.
- In an arid region where terrestrial DOC input is low (<0.3 g/m²-yr), controlling reservoir hydraulic retention time (HRT) is key to reducing the amount of DOC production and export to downstream.
Culprit Algae can be cultured in the lab to study environmental factors

- Increasing light intensity affects biomass production and yield of T&O compounds
- Intracellular MIB and geosmin levels are much higher than concentrations in aqueous media
Raw water entering 24th Street WTP from a concrete lined canal

- MIB
- Geosmin
- Beta-cyclocitrinal

Concentration (ng/L)

Year

1999 2000 2001 2002 2003 2004
Powder Activated Carbon can remove T&O compounds within WTPs

![Graph showing MIB Concentration (ng/L) from 1999 to 2004 for Raw water and Finished water.]

Relationships between FPA and GC/MS Analysis

![Graph showing the relationship between Cyclocitral ng/L and Earthy/Musty FP. The equation is y = 0.1865x + 1.5136 with R^2 = 0.0801.]]
Predicting Expected FPA Intensity based upon GC/MS data

Earthly Musty FPA Value = 0.800*MIB^{0.396}*Geo^{-0.110}*Cyclocitral^{0.350}

R^2 = 0.728

FPA= 2 when MIB=5, Geosmin=3, Cyclocitral=3 ng/L

Cyanotoxins are also present (ng/L to ug/L)

How can we make toxin analysis WTP friendly?

<table>
<thead>
<tr>
<th>Toxin groups</th>
<th>Chemical Structure</th>
<th>Primary target organ in mammals</th>
<th>Cyanobacterial genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystins</td>
<td>Cyclic peptides</td>
<td>Liver</td>
<td>Microcystis, Anabaena, Oscillatoria, Nostoc</td>
</tr>
<tr>
<td>Nodularin</td>
<td>Cyclic peptides</td>
<td>Liver</td>
<td>Nodularia</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>Alkaloid</td>
<td>Liver</td>
<td>Cylindrospermosis Anabaena Aphanizomenon Umezakia</td>
</tr>
<tr>
<td>Anatoxin-a, Anatoxin-a (S)</td>
<td>Alkaloid</td>
<td>Nerve synapse</td>
<td>Anabaena, Planktothrix, Aphanizomenon, Cylindrospermopsis</td>
</tr>
<tr>
<td>Saxitoxins</td>
<td>Alkaloid</td>
<td>Nerve axons</td>
<td>Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis</td>
</tr>
</tbody>
</table>
ELISA
(enzyme-linked immunosorbent assay)

Step #1 Well is coated with antibody (Y)

Step #2 Sample is added and microcystin (♂) is bound by the antibody (Y)

Step #3 Enzyme linked antigen (♂) is added and binds to the remaining unoccupied sites on the antibody (Y)

Step #4 Enzyme substrate (♂ ) is added to produce color reaction

ELISA Calibration Curve

%Bo = -26.72 Ln [MC] + 40.13
R² = 0.9976

Microcystin Concentration [MC] (µg/L)

%Bo = OD sample x 100%

OD neg control
### ELISA Assay Results

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Date</th>
<th>Kit No.</th>
<th>50%Bo (µg/L)</th>
<th>Slope</th>
<th>R²</th>
<th>Acceptable</th>
<th>Range (µg/L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/27/02</td>
<td>1</td>
<td>0.4</td>
<td>-28.8</td>
<td>0.957</td>
<td>Yes</td>
<td>0.16-1.6</td>
<td>Calibrators less than recommended %Bo ranges. Near expiration date</td>
</tr>
<tr>
<td>2</td>
<td>1/22/02</td>
<td>1</td>
<td>0.2</td>
<td>-22.3</td>
<td>0.947</td>
<td>Yes</td>
<td>0.16-1.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2/02/02</td>
<td>2</td>
<td>0.2</td>
<td>-22.7</td>
<td>0.961</td>
<td>Yes</td>
<td>0.16-1.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5/15/02</td>
<td>2</td>
<td>0.2</td>
<td>-24.3</td>
<td>0.947</td>
<td>Yes</td>
<td>0.16-1.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
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<td>2</td>
<td>0.5</td>
<td>-28.3</td>
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<td>Yes</td>
<td>0.16-1.6</td>
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<tr>
<td>6</td>
<td>8/28/02</td>
<td>3</td>
<td>0.6</td>
<td>-27.0</td>
<td>0.993</td>
<td>Yes</td>
<td>0.16-1.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
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<td>3</td>
<td>0.6</td>
<td>-24.1</td>
<td>0.975</td>
<td>Yes</td>
<td>0.16-2.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9/19/02</td>
<td>4</td>
<td>0.6</td>
<td>-22.6</td>
<td>0.989</td>
<td>Yes</td>
<td>0.16-2.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10/10/02</td>
<td>5</td>
<td>0.6</td>
<td>-27.6</td>
<td>0.992</td>
<td>Yes</td>
<td>0.16-2.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10/17/02</td>
<td>6</td>
<td>0.6</td>
<td>-26.7</td>
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<td>Yes</td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>10/25/02</td>
<td>7</td>
<td>0.6</td>
<td>-26.8</td>
<td>0.987</td>
<td>Yes</td>
<td>0.16-2.5</td>
<td></td>
</tr>
</tbody>
</table>

### PP2A (protein phosphatase-2A assay)

- **Step #1** Well is coated with enzyme PP1 or PP2A (○).
- **Step #2** Sample is added and microcystin (●) inhibits enzyme.
- **Step #3** Substrate (●) is added. Over time, inhibited enzyme reacts with substrate releasing phosphate (PO₄³⁻) resulting in yellow color. Enzymes inhibited by microcystin do not react with substrate and produce no color.
PP2A Assay Results

<table>
<thead>
<tr>
<th>Assay No.</th>
<th>Date</th>
<th>Enzyme No.</th>
<th>V/Vo=50% (µg/L)</th>
<th>Slope</th>
<th>R²</th>
<th>Acceptable</th>
<th>Assay Range (µg/L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/26/02</td>
<td>1</td>
<td>0.15</td>
<td>-3.12</td>
<td>0.9988</td>
<td>Yes</td>
<td>0.05-0.30</td>
<td>Enzyme at room temperature</td>
</tr>
<tr>
<td>2</td>
<td>6/06/02</td>
<td>1</td>
<td>0.15</td>
<td>-3.36</td>
<td>0.9415</td>
<td>Yes</td>
<td>0.05-0.30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6/13/02</td>
<td>1</td>
<td>0.15</td>
<td>-3.03</td>
<td>0.9143</td>
<td>Yes</td>
<td>0.05-0.30</td>
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</tr>
<tr>
<td>4</td>
<td>6/20/02</td>
<td>2</td>
<td>0.15</td>
<td>-2.95</td>
<td>0.7110</td>
<td>Yes</td>
<td>0.05-0.625</td>
<td>Poor R² value</td>
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<tr>
<td>5</td>
<td>6/27/02</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>0.2480</td>
<td>No</td>
<td>NA</td>
<td>New enzyme used, calibrators not in range of Vi/Vo &lt; 1</td>
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<td>0.3110</td>
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</tr>
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<td>NA</td>
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<td>8</td>
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<td>0.6</td>
<td>-0.54</td>
<td>0.9276</td>
<td>Yes</td>
<td>0.2-1.2</td>
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<td>3</td>
<td>0.8</td>
<td>-0.55</td>
<td>0.9741</td>
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<td>0.2-1.2</td>
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<td>3</td>
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<td>-0.55</td>
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<tr>
<td>11</td>
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<td>0.7</td>
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<td>0.8329</td>
<td>Yes</td>
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</tr>
<tr>
<td>12</td>
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<td>3</td>
<td>0.7</td>
<td>-0.52</td>
<td>0.9626</td>
<td>Yes</td>
<td>0.2-1.5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7/24/02</td>
<td>3</td>
<td>0.9</td>
<td>-0.55</td>
<td>0.9380</td>
<td>Yes</td>
<td>0.2-1.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>8/02/02</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>NA</td>
<td>Enzyme was inactive from overuse at room temperature</td>
</tr>
<tr>
<td>15</td>
<td>8/02/02</td>
<td>4</td>
<td>1.1</td>
<td>-0.55</td>
<td>0.9027</td>
<td>Yes</td>
<td>0.2-1.6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>8/02/02</td>
<td>4</td>
<td>0.6</td>
<td>-1.05</td>
<td>0.9297</td>
<td>Yes</td>
<td>0.2-6.9</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>8/21/02</td>
<td>4</td>
<td>1.4</td>
<td>-0.43</td>
<td>0.9588</td>
<td>Yes</td>
<td>0.3-1.8</td>
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</tr>
<tr>
<td>18</td>
<td>8/28/02</td>
<td>4</td>
<td>1.0</td>
<td>-0.47</td>
<td>0.9917</td>
<td>Yes</td>
<td>0.2-1.5</td>
<td></td>
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<tr>
<td>19</td>
<td>9/12/02</td>
<td>4</td>
<td>1.0</td>
<td>-0.55</td>
<td>0.9310</td>
<td>Yes</td>
<td>0.4-1.5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9/19/02</td>
<td>5</td>
<td>1.5</td>
<td>-0.19</td>
<td>0.7516</td>
<td>No</td>
<td>0.5-2.5</td>
<td>Inconsistent calibrator results</td>
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<td>21</td>
<td>9/25/02</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>NA</td>
<td>No color produced</td>
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<tr>
<td>22</td>
<td>10/03/02</td>
<td>4</td>
<td>1.2</td>
<td>-0.36</td>
<td>0.9416</td>
<td>Yes</td>
<td>0.5-2.1</td>
<td></td>
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<tr>
<td>23</td>
<td>10/03/02</td>
<td>4</td>
<td>1.4</td>
<td>-0.55</td>
<td>0.9077</td>
<td>Yes</td>
<td>0.5-2.5</td>
<td></td>
</tr>
</tbody>
</table>

Assay Comparison

MC concentration (µg/L)

ELISA

PP2A

1:1
Assay Comparison

<table>
<thead>
<tr>
<th></th>
<th>ELISA (presence)</th>
<th>PP2A (toxicity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDL (mg/L)</td>
<td>0.147</td>
<td>0.05, 0.24</td>
</tr>
<tr>
<td>Range (ug/L)</td>
<td>0.147-2.5</td>
<td>0.05-0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.24-2.5</td>
</tr>
<tr>
<td>% acceptable</td>
<td>100%</td>
<td>74%</td>
</tr>
<tr>
<td>Cost per well</td>
<td>$4.12</td>
<td>~$1.00</td>
</tr>
</tbody>
</table>

- Recommend ELISA for monitoring

Intracellular Extraction

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Microcystis aeruginosa (µg-MC/OD&lt;sub&gt;730&lt;/sub&gt;)</th>
<th>24 hrs in MIOH + Beadbeating</th>
<th>24 hrs in MIOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs in MtOH + Beadbeating 10 day</td>
<td>24 hrs in MtOH 29 day</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.662</td>
<td>1.042</td>
<td>0.437</td>
</tr>
<tr>
<td>2</td>
<td>0.732</td>
<td>1.076</td>
<td>0.380</td>
</tr>
<tr>
<td>3</td>
<td>0.634</td>
<td>0.991</td>
<td>0.408</td>
</tr>
<tr>
<td>4</td>
<td>0.915</td>
<td>0.762</td>
<td>0.775</td>
</tr>
<tr>
<td>5</td>
<td>0.887</td>
<td>0.758</td>
<td>0.648</td>
</tr>
<tr>
<td>6</td>
<td>0.859</td>
<td>0.275</td>
<td>0.592</td>
</tr>
<tr>
<td>7</td>
<td>0.242</td>
<td>0.275</td>
<td>1.056</td>
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<tr>
<td>8</td>
<td>0.275</td>
<td>0.944</td>
<td>0.874</td>
</tr>
<tr>
<td>9</td>
<td>0.958</td>
<td>0.958</td>
<td>0.829</td>
</tr>
</tbody>
</table>

Mean: 0.782 0.926 0.689 0.881

Standard Deviation: 0.121 0.154 0.257 0.116

Culture Growth = 10 days  Culture Growth = 29 days
Extracellular conc. = >2.5 µg-MC/L  Extracellular conc. = >25 µg-MC/L
Intracellular conc. = 56 µg-MC/L  Intracellular conc. = 838 µg-MC/L
Microcystin Monitoring

Intracellular levels always slightly higher than extracellular (dissolved) MC concentrations

MIB and MC
Lake Pleasant
Cyanotoxin Summary

- Relatively large background levels of microcystin found in all wastesheds.
- Anatoxin-a only found in fish stomach samples, however, the likely cause of large fish kills.
- Fast degradation rates of anatoxin-a make this neurotoxin extremely difficult to detect in surface water.
- While numbers of *C. raciborskii* have, at times, dominated the phytoplankton cylindrospermopsin has been found in very low levels and only in concentrated samples (plankton tows).

Early warning indicators for culprit algae
General strategy

Field sample → DNA extraction & purification → PCR amplification of 16S rDNA fragments → genomic DNA → Database matching → Species identification → Field PCR products → DGGE separation → cyanobacterial fingerprints

DNA from an individual species → PCR amplification → DNA extraction from the gel

PCR amplification

AGCTCCATTAGGGA
C
TCGAGGTAATCCGTG

DNA sequencing

Species identification

Relation between DNA profiles and MIB throughout the seasons

DNA marker

Time of the year (2002)

MB (mg L⁻¹)

0 5 10 15 20 25 30

5/15 7/29 8/12 8/26 9/30 10/14 10/28 11/12 11/25

DNA from an individual species
Relation between DNA profiles and MIB at selected sites

A

B

C

DNA marker

Pima Rd through 29th Ave (8/28/02)

Sampling sites

MIB (ng L⁻¹)

Before Cu²⁺ treatment

After Cu²⁺ treatment

MIB (ng L⁻¹)

Sampling sites

Before Cu²⁺ treatment

After Cu²⁺ treatment
Detection of *Cylindrospermopsis* in Saguaro Lake samples using a specific gene probe

1: *Anabaena* TAC426  
2: Saguaro lake sample (6/15/04)  
3: *Nodularia* strain 575  
4: *Cylindrospermopsis* AWT205  
5: *Plankothrix* PCC7811  
6: *Microcystis* LE-3  
7: *Nostoc* PCC73102  
8: Saguaro lake sample (6/22/04)  
9: *Aphanizomenon* strain Zayi  
10. No DNA sample  

100 bp DNA ladders used at both sides of the sample lanes.

Summary

- Algae metabolites include:  
  - Bulk OM (mg/L) that produces THMs and is enriched in organic nitrogen  
  - T&O terpenoids (ng/L) with high intracellular levels. Produced by cyanobacteria with specific genetic fingerprints, but which represent a small percentage of the total algae biomass  
  - Cyanotoxins (ug/L) are present, have lead to fish kills (makes news headlines), and has raised concern by many WTPs  
  - 16S rDNA primers have resolved 16 different cyanobacterial sequences from the AZ Canal by PCR and DGGE techniques  
- Variable hydrologic conditions, reservoir management, and water conveyance will affect magnitude for the significance of algae metabolites in Arizona  
- T&O and cyanotoxins can be removed in WTPs, but provide added motivation for WTPs to install GAC  
- #1 problem reported by each WTP related to algae: how to control inplant algae growth without prechlorination. So this is our challenge for 2005  
- Arizona watersheds are representative of many others throughout the western US
Paul Westerhoff, Ph.D.
Arizona State University
Associate Professor
Department of Civil & Environmental Engineering
http://ceaspub.eas.asu.edu/pwest/

Acknowledgements:
AwwaRF
City of Phoenix
City of Tempe
City of Peoria
Salt River Project
Central Arizona Project
Michelle Cummings, MyLinh Nguyen, Mario Esparza-Soto
ASU – NSF Water Quality Center