Quantifying Moss Response to Contaminant Exposure Using Laser Induced Fluorescence

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Introduction and Motivation

• TA2: Develop Technology and Methodology Using and Observing Biota

• Goal: Detect contamination - nuclear fallout, mining waste, metals, organics, etc. through its accumulation in biota

• Project developing a non-invasive, remote laser induced fluorescence (LIF) detection technique using the Biofinder (Misra et al., 2018), currently focusing on moss
  • Biofinder characterizes biological material through LIF
  • Moss is robust, simplistic species, used extensively in environmental monitoring – atmospheric accumulation of metals
  • BUT traditional approaches collecting and analyzing moss are labor intensive and destructive – need for remote sensing approach

• Hypotheses:
  • Metal deposition induces a physiological response in moss
  • Response from moss can be detected using LIF
  • Images of LIF can be analyzed to detect/identify contamination

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Mission Relevance

• The NNSA mission is focused on detection and prevention of the militarization of nuclear weapons

• The work presented aims to develop a non-invasive remote technique to detect biological response in vegetation after its exposure to metals of interest from:
  • Nuclear event
  • Mining waste
  • Nuclear waste

• End goal: Develop remote sensing technology to survey areas of interest to:
  • Produce maps of metal/contamination spatial distribution and pin-point its source
  • Identify the presence and type of contamination
  • Aid in bioremediation
Technical Approach – Part 1

• In collaboration with Wendy Kuhne and Laura Tovo at Savannah River National Laboratory

• Two Part Methodology:
  • Part 1: Cultivate moss, dose with metal of interest (CuCl₂)
    • 10-day experiment, 1 control, 3 treated moss mats
    • 5 doses of copper chloride treatment (once every 48-hrs)

<table>
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<th>Day:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>0.54</td>
<td>1.08</td>
<td>1.08</td>
<td>1.62</td>
<td>1.62</td>
<td>2.16</td>
<td>2.16</td>
<td>2.70</td>
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<tr>
<td>Trial 3</td>
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<td>1.08</td>
<td>2.16</td>
<td>2.16</td>
<td>3.24</td>
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<tr>
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<td>1.62</td>
<td>3.24</td>
<td>3.24</td>
<td>4.86</td>
<td>4.86</td>
<td>6.48</td>
<td>6.48</td>
<td>8.10</td>
<td>8.10</td>
</tr>
</tbody>
</table>

*table displays cumulative Cu concentration in μmol/cm²

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Technical Approach – Part 2

• Part 2: LIF Imaging using Biofinder and Data Analysis
  • Cu treated moss samples were imaged under LIF
    • Every 6-hrs for first 48-hrs, then every 24-hrs
  • Daylight imaging at .5 m distance using CMOS Camera
    • 532 nm green laser and 355 nm UV laser (Nd:Yg pulsed lasers)
  • Images collected for both lasers and each laser individually
  • Images processed in MATLAB – density histograms created from pixel count
Technical Approach – Part 2 cont.

- Day-0 used as reference for comparison
- Shown here is Day-0 of Trial 4 to provide an example

- Each image from each trial is compared to its Day-0 image
- Shown here is Day-10 of Trial 4 to provide an example

Density Histogram Differences

Dynamic Time Warping

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Results – 48 Hour Imaging Analysis

- Biofinder imaging within 5 minutes of dosing already produces measurable response
- All treated trials clear control average ± 2σ over 48-hours, never (within 48-hrs) return to pre-treatment level
- Curves show immediate response followed by steady increase and flattening

Cu in moss after day 1 treatment [μmol/g]:
- Trial 1: 0
- Trial 2: 21 ± 10
- Trial 3: 47 ± 13
- Trial 4: 70 ± 13
Results – 10-Day Imaging Analysis

• One color analysis
  • With increased Cu dose shift to “darker,” lower RGB decimal code values
  • Curves more peaked, more right skewed

• Two color analysis
  • Relationship between red and green color channels – shapes change with Cu dose
  • Increased Cu toxicity leads to a longer, narrower “loop” as red and green become more similar, right skewed, and peaked
Results – 10-Day Imaging Analysis cont.

• Density Histogram Difference
  • Intersection difference similar between color channels
  • Increased difference with Cu dose
  • Curves show steep initial response followed by steady increase

• Dynamic Time Warping (DTW)
  • Alternative method for curve comparison
  • Similar to intersection difference
  • Increase in distance equal to separation from day-0 with increasing Cu

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Results – One Color vs. Two Color Analysis

- Trial 1 (control) closely clustered around its 10-day mean
- All treated trials above average $\pm 3\sigma$ of trial 1 starting day-1 treatment
- Appears multiple small or single large dose result in the same difference
- Two-color analysis appears to show greater sensitivity than one-color analysis
- Same trend between curves, greater separation from control average $\pm 3\sigma$ using DTW (two-color analysis)
Expected Impact

• Promise of laser methodology for field application
• Real-time detection of metal contamination in biota
• Eliminate need for extensive sampling & allows targeted sampling and laboratory analysis
• Help identify source of contamination and its distribution
• Not limited by time of day (as opposed to NIR)
• Low energy use – portable in current state
  • Further development of programming and housing needed
• End goal is remote sensing via drone
MTV Impact

• MTV currently supports one graduate, and two undergraduate students and has allowed for collaboration with national laboratories
  • Co-developed experimental design
  • Discussed future collaborations, opportunities for site visits and internships
  • Next-Gen AI for Proliferation Detection: Domain Aware Methods Workshop
  • NSSC Alumni Speaker Series
• Potential technology field application - Savannah River National Laboratory
• Other collaboration and internship opportunities discussed - Pacific Northwest National Laboratory, Lawrence Livermore National Laboratory
Conclusion

- LIF is a viable remote sensing method for detecting metal exposure in moss:
  - Moss physiological response to Cu results in measurable changes in LIF using the “Biofinder”
  - Single-color (red) and two-color (red and green) analyses of treated sample images show separation from control - All treated trials above 3σ of trial 1 control mean
  - This was a proof-of-concept study, lowest dose (340 mg/m²) detection sensitive enough for extremely contaminated sites (Murgul Cu mine, Turkey) but need to improve sensitivity to <1 mg/m² to monitor environmental levels

- Preliminary data on other metals (Zn, Pb) showed promise, U method in development

- Continued work towards improved sensitivity will make methods field applicable
  - work with National Labs to establish desired sensitivity levels

- Develop methods for real-time processing, remote sensing, and field tests (e.g., at SRNL)

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Next Steps

• Improved Sensitivity of Biofinder
  • Detection in the 1-10 nmol/cm\(^2\) range needed for environmental levels
  • Establish sensitivity-to-distance relationship, optimize remote sensing

• Test Biofinder with other metals and biota
  • Do full experiments with Cu, Zn, and Pb; Begin development for U experiments
  • Application to other vegetation (bioaccumulators)
  • Chlorophyll tests to determine chl-a/b ratios effect on images
  • Moss and other plant stress tests: environmental, temperature, nutrients, etc.

• Undergraduate projects:
  • Cultivation of moss and new plants
  • Image analysis (moss mats vs fronds, other plants)
  • Chlorophyll analysis

• Foster Collaborations with National Labs

• Further Develop Machine Learning Technique
  • Assist with real time image analysis and contaminant identification
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