Comparison of two laser systems for monitoring physiological changes in moss (Thuidium plicatile) due to metal contamination using Laser-Induced Fluorescence (LIF)

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Introduction and Motivation

Development of non-invasive, in-situ detection methods are of major interest to environmental monitoring of heavy metals and radionuclides. Anthropogenic activities such as mining and industrial production may introduce a high degree of contaminants to the environment that can affect ecosystems near the source. Laser-Induced Fluorescence (LIF) is a non-destructive technique that can be used to quantify physiological change due to changes in chlorophyll a and b ratios in plants. Moss have become suitable environmental indicators because of their ability to accumulate and absorb pollutants in an environment (Berg & Steinnes, 1997; Degola et al., 2014). Pairing moss and LIF can provide useful information about mechanisms contributing to stress responses from heavy metal and radionuclide contamination. Our objective is to test two laser systems: the Standoff Biofinder used in previous work (Kelly, 2020) and a new laser system aimed at targeting chlorophyll a and b excitation. The final goal is to determine the best parameters for application to a field ready system that can detect contamination in the environment.

Mission Relevance

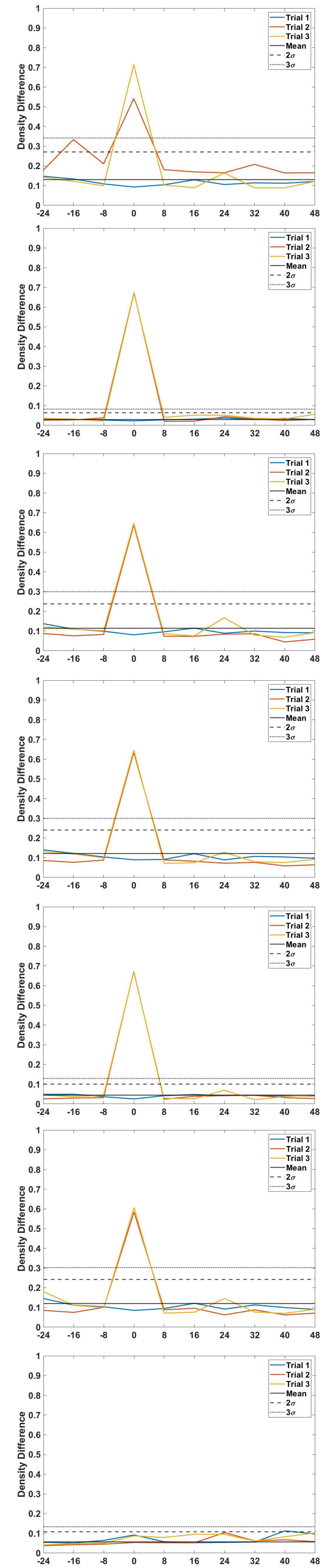
The NNSA Mission aims to detect and prevent threats of nuclear destruction by improving overall safety and security of nuclear weapons and radioactive material. Efforts to best monitor and identify the sources of heavy metal contamination and deposition of radioisotopes is imperative in extraction and removal of these pollutants. This project aims to contribute to the development of a remote-sensing system based on LIF application to vegetation in the field at a site of interest.

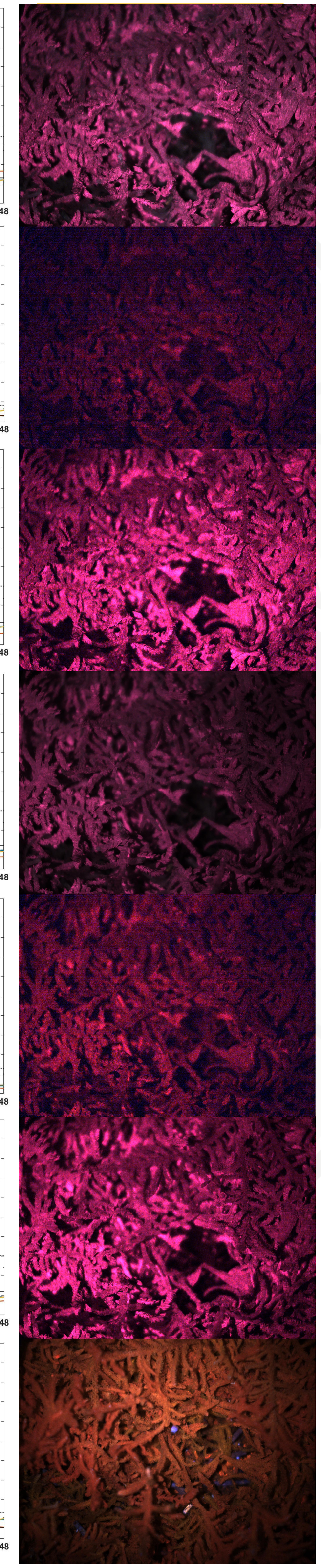
Technical Approach

Moss was selected and cultivated under greenhouse conditions prior to treatment to provide an acclimation period. 3 moss trays were treated with known, increasing levels of Cu concentrations (1 nmol/cm2 , 10 nmol/cm2 , 100 nmol/cm2) after initial imaging collected control baselines for each sample. Physiological response due to metal toxicity were measured using LIF every 8 hours. The lasers with known wavelengths, 445 nm and 462 nm, correspond to chlorophyll a and b absorption peaks in the blue spectra of visible light. Images were collected for each laser without a filter and with 650 nm and 670 nm bandpass filters. These measurements were compared with those using the Standoff Biofinder, which has lasers at 355 nm and 532 nm (Misra et al., 2018).

The degree of LIF response is captured as an image using a CMOS camera and quantified by changes in the RGB pixel decimal codes between control and treated moss images. The decimal codes are used to create histograms showing the abundance of each color channel for each trial. These histograms can then be used for direct comparison to the control based upon their degree of overlap or difference between curves.

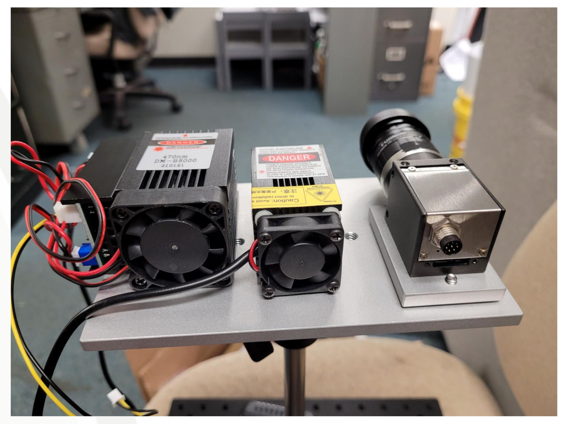
Chlorophyll A - 445 nm: w/o filter, 650nm, 670nm
Chlorophyll B – 462 nm: w/o filter, 650nm, 670nm
Biofinder: 532 nm + 355 nm

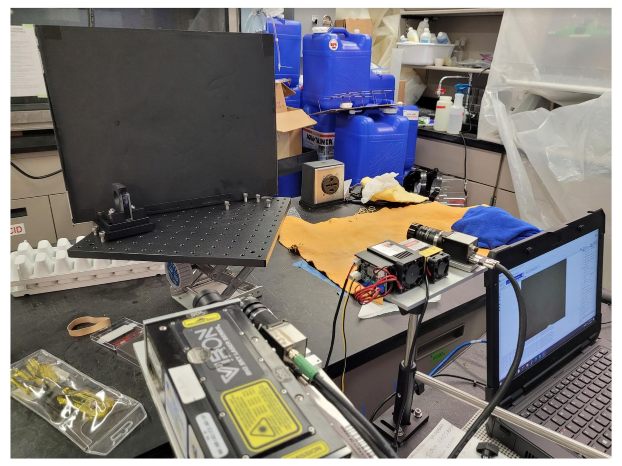




Results & Expected Impact

Comparison of the control and treated moss using the density difference and DTW histograms suggests that the blue channel is least affected by the natural variability of the plant and thus better at detecting when the moss has been treated with Cu. Detection using the chl a/b lasers appears most conclusive when using their respective filters. Physiological changes at the 10 and 100 nmol/cm2 scale are detected through image analysis of the chlorophyll specific lasers, but the Biofinder does not detect these levels. All figures shown are only of the blue channel. Utilization of a laser system with specific wavelengths (445 nm or 462 nm) corresponding to chlorophyll a or b absorption peaks can improve sensitivity of the LIF technique while also allowing for a more cost effective and portable system for remote sensing application in the field.





MTV Impact

MTV provides the opportunity for both an undergraduate and graduate student to build on meaningful work in collaboration with the national laboratories. Dr. Anupam Misra from University of Hawaii at Manoa granted access to the Biofinder and Dr. Henrietta Dulai assisted in experimental work for the trials. This project will provide knowledge on detection methods that can improve environmental monitoring. Savannah River National Laboratory has helped guide the focus regarding vegetation and potential application to bioremediation since the inception of the project. Lawrence Livermore National Laboratory offered expertise on laser system design, calibration, and experimental design for the chlorophyll specific experiments.

Conclusion & Next Steps

Analysis of LIF images produced from the chlorophyll experiment show that use of specific laser wavelengths for monitoring changes in chlorophyll due to contamination is viable. Previous work with the Biofinder could only detect Cu levels at 100 nmol/cm2. after successive increasing doses. The 445 nm and 462 nm lasers, however, show immediate identification of metal contamination at the 10 nmol/cm2 level with a single dose. Future work will focus on other plant types, metal and stress conditions, and the application and challenges of further developing these lasers for a portable field-ready remote sensing system.

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