Chlorophyll extract measurements using Laser-Induced Fluorescence (LIF) of moss (*Thuidium plicatile*) in response to copper contamination

Ciara Bongolan-Aquino, Kelly Truax, and Henrietta Dulai

University of Hawaiʻi at Mānoa

caquino6@hawaii.edu

Consortium for Monitoring, Technology, and Verification (MTV)

Introduction and Motivation

Anthropogenic activities contaminate the environment with heavy metals and radionuclides that may be taken up by vegetation (Berg & Steinnes, 1997; Degola et al., 2014). Laser-Induced Fluorescence (LIF) can quantify physiological changes in plants due to metal stress by analyzing responses in a plant's chlorophyll content. In previous work, LIF was used to track a plant's stress response to heavy metal contamination in moss mats (Truax, 2020). The key motivation of this project is to utilize extracted metal and chlorophyll measurements from metal dosed plants and evaluate the timing and range of chlorophyll changes. The project helps in the development of a non-invasive, in-situ LIF detection method that can locate areas contaminated by heavy metals.

Mission Relevance

The development of methods to monitor and identify clandestine activities involving metals and radionuclides is one of NNSA's missions. This project's contribution to this mission is the development of a LIF-based remote-sensing system for environmental monitoring of metals bioaccumulated in vegetation.

Technical Approach

Thuidium plicatile is an endemic moss species of Hawaii. Samples were collected from the Wa'ahila area on O'ahu and cleaned before incubation on trays. Trays were dosed with copper in order to induce physiological response in the moss and imaged using LIF. In the next step, chlorophyll and copper were extracted from the plants.

LIF spectroscopy and spectrophotometry of chlorophyll extracts and ICP-MS analysis of extracted copper was conducted every 24 hours within a four-day period. The changes in LIF response to stress was captured as an image with a CMOS camera. The control baseline LIF images were collected at -24 hours. Copper was administered at 0 hours. LIF images were collected at 0, 24, and 48 hours after heavy metal contamination.

Changes in the images' RGB pixel decimal codes between the control (-24 hours) and subsequent images after copper dosing were then used to quantify the stress response. A comparison of the LIF images of plants and extracted chlorophyll as well as extracted metals helped identify best approaches to identify copper in plants and characterize the method's sensitivity.

Figure 1: Lasers and camera (left). LIF imaging setup (right).

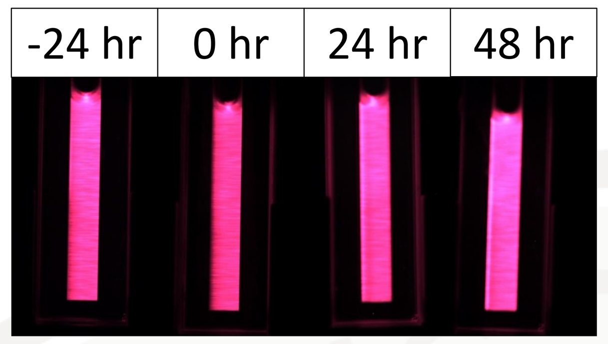


Figure 2: LIF of chlorophyll extracts from -24, 0, 24 and 48 hours with respect to dosing captured with a chlorophyll sensitive laser (Truax, 2023).

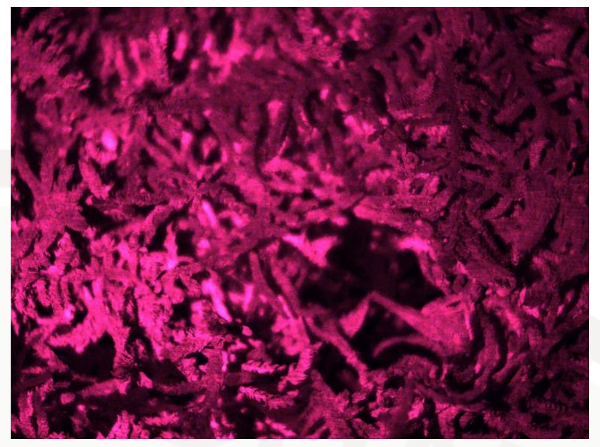


Figure 3: *Thuidium plicatile* under LIF.

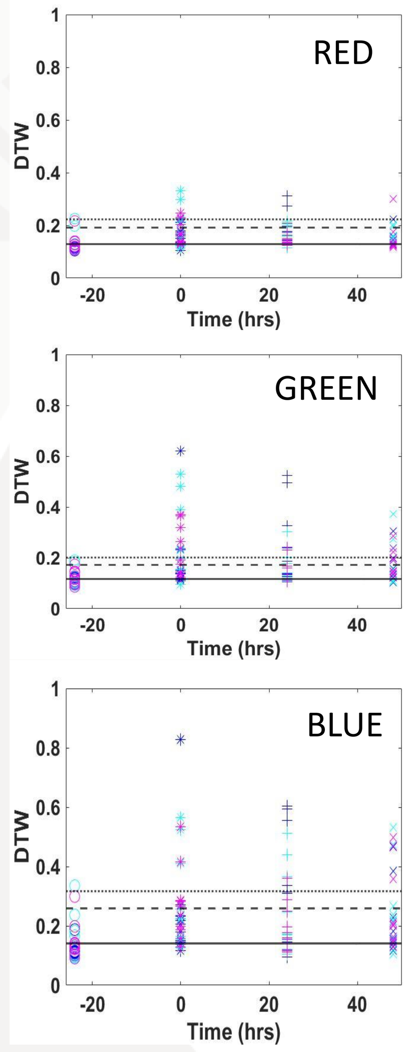


Figure 4: Comparison of single colors: red, green, and blue from LIF images of chlorophyll extracts obtained at -24, 0, 24 and 48 hours to a control using distance (DTW) analysis.

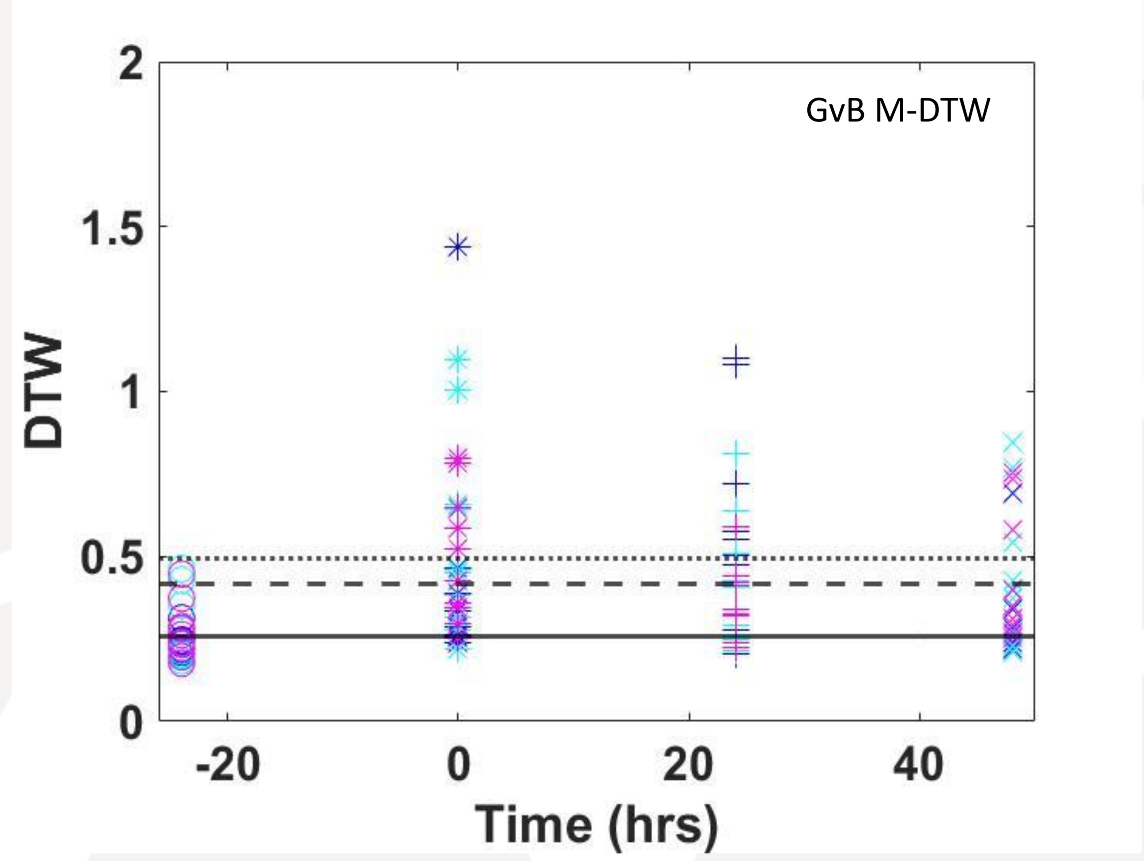
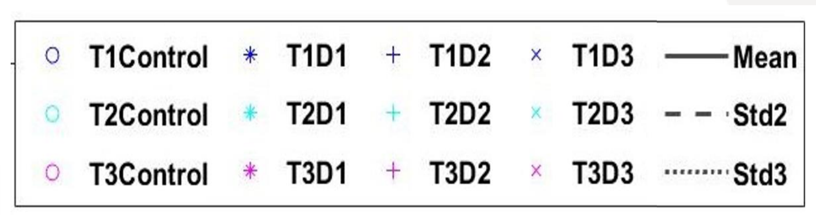


Figure 5: A Multi-color DTW analysis that compares green and blue color channels from LIF images of chlorophyll extracts.



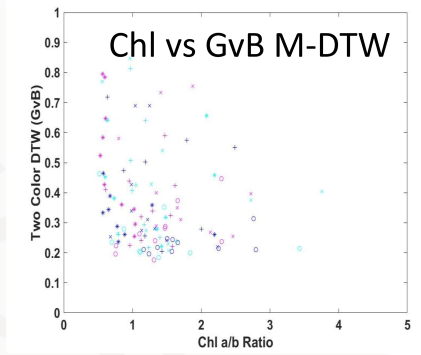


Figure 6: A plot comparing the chlorophyll extract results to the GvB Multi-color DTW results.

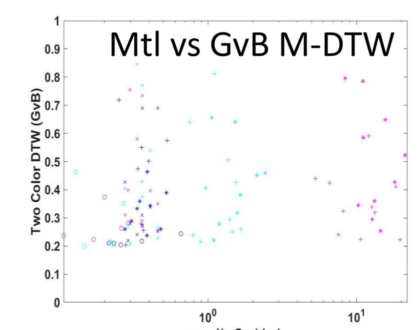


Figure 7: A plot comparing the metal extract results to the GvB Multi-color DTW results.

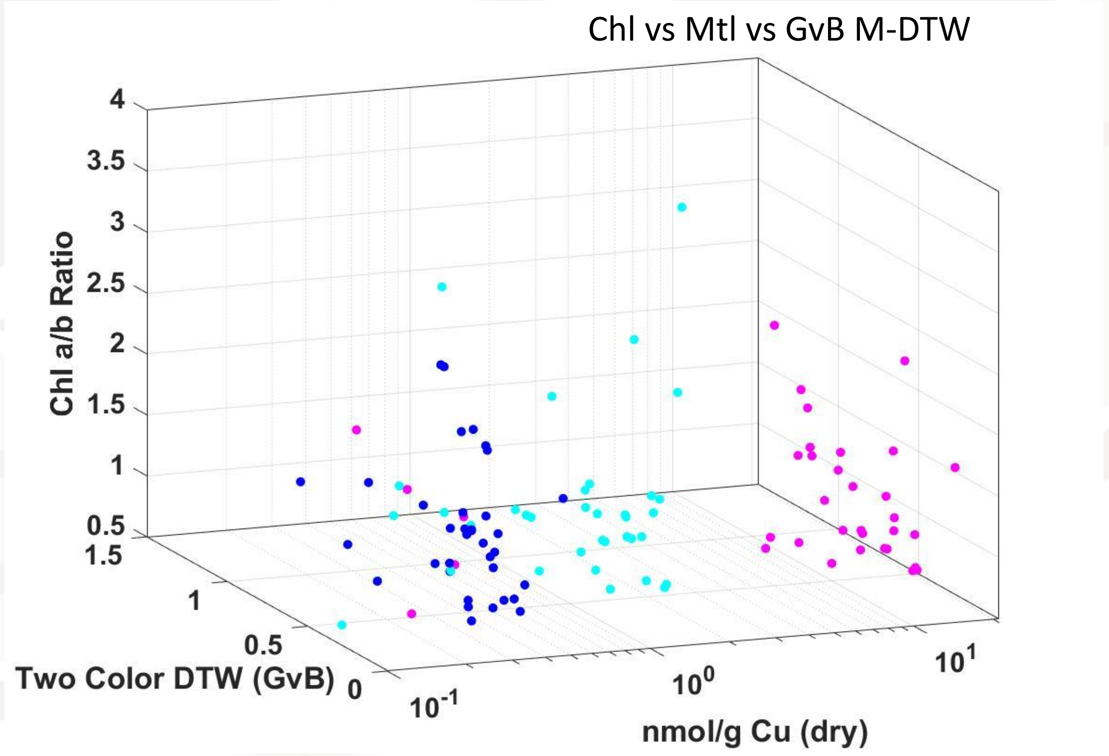


Figure 8: A 3D plot comparing the chlorophyll extract, metal extract, and GvB Multi-color DTW results.

Results & Expected Impact

LIF analysis of chlorophyll extracts shows that images taken -24 hours were indistinguishable from the control, images following copper dosing at 0 hours had the largest response in chlorophyll change, which then tapered off but persisted throughout the 48 hours when the last images were taken. We were able to link the variation in RGB decimal code values to changes in chlorophyll A/B ratios and correlated them to levels of heavy metal contamination. This methodology can be used to determine whether a plant in the field has been contaminated by copper and quantify the amount of copper that has accumulated in the plant.

MTV Impact

This project provided learning opportunities in MatLab coding and LIF applications under the supervision of Dr. Kelly Truax. Dr. Henrietta Dulai gave me the opportunity to work on this project and introduced me to the goals of MTV.

The Savannah River National Laboratory has led the helm in applying vegetation in bioremediation efforts. The Lawrence Livermore National Laboratory has given guidance on laser system design, calibration, and experimental design for experiments that are focused on chlorophyll analysis.

Conclusion & Next Steps

The analysis of LIF images through variations in RGB decimal code values has been shown to be linked to changes in chlorophyll A/B ratios and correlated to levels of heavy metal contamination. This project developed the groundwork for the application of Laser-Induced Fluorescence (LIF) for real-time analysis of extracted chlorophyll and whole plants. Further development of this method will aid in identifying plants contaminated by various heavy metals and aid in environmental monitoring.

References

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