Comparison of two laser systems used in a laser-induced fluorescence (LIF) method to detect physiological changes in *Azolla filiculoides* due to lead exposure

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Introduction and Motivation

Deposition of heavy metals and radionuclides from anthropogenic activities such as mining and industrial production have raised concern for human health and environmental safety. These contaminants can be stored within a plant's tissue and induce physiological changes by altering its metabolism. Development of a non-invasive detection method has gained much interest in monitoring contaminants within the environment. Laser-induced fluorescence (LIF) spectroscopy has shown real-time analysis of chlorophyll by quantifying molecular energy level change due to laser excitation that produces fluorescence as a biological response. These responses can be captured by a CMOS camera in which pixels are extracted and analyzed as histograms to show the change in RGB decimal code values. These histograms demonstrate the strong correlation between changes in total chlorophyll content and metal uptake. Two laser systems with wavelengths of 445 nm and 462 nm corresponding to chlorophyll a and b absorption peaks were used, respectively. This project used *Azolla filiculoides* as the study plant due to its history of extraction and removal of contaminants in aqueous environments. Testing distances and light intensities to image Azolla with various levels of lead contamination help enhance this remote sensing technique in identification and quantification of heavy metals concentrations within the environment.

Mission Relevance

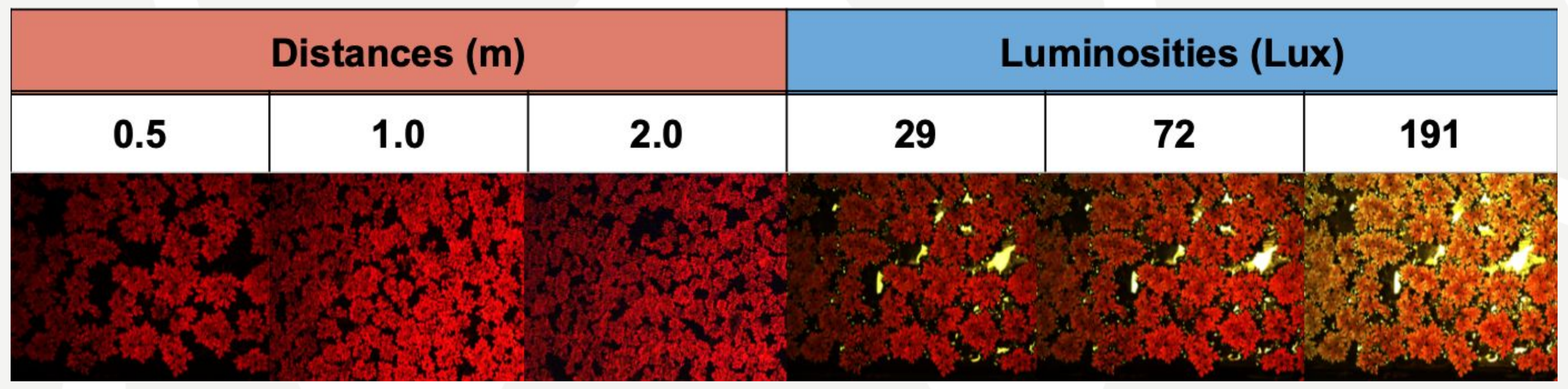
MTV's mission is to develop new technologies that can detect and deter nuclear proliferation activities, among others by detecting schemes that would contribute to foreign nuclear weapons development programs. Being able to identify environmental fingerprints of such activities such as the presence of certain radionuclides and metals in biota therefore directly contributes to this mission. This project builds on previous work that successfully demonstrated the application of laser induced fluorescence (LIF) to detect metals absorbed by biota, by testing LIF system sensitivity as a remote-sensing system.

Expected Impact

This work helps fine tune laser system settings and explore its capabilities to further enhance this remote sensing technique in identification and quantification of heavy metals concentrations within the environment.

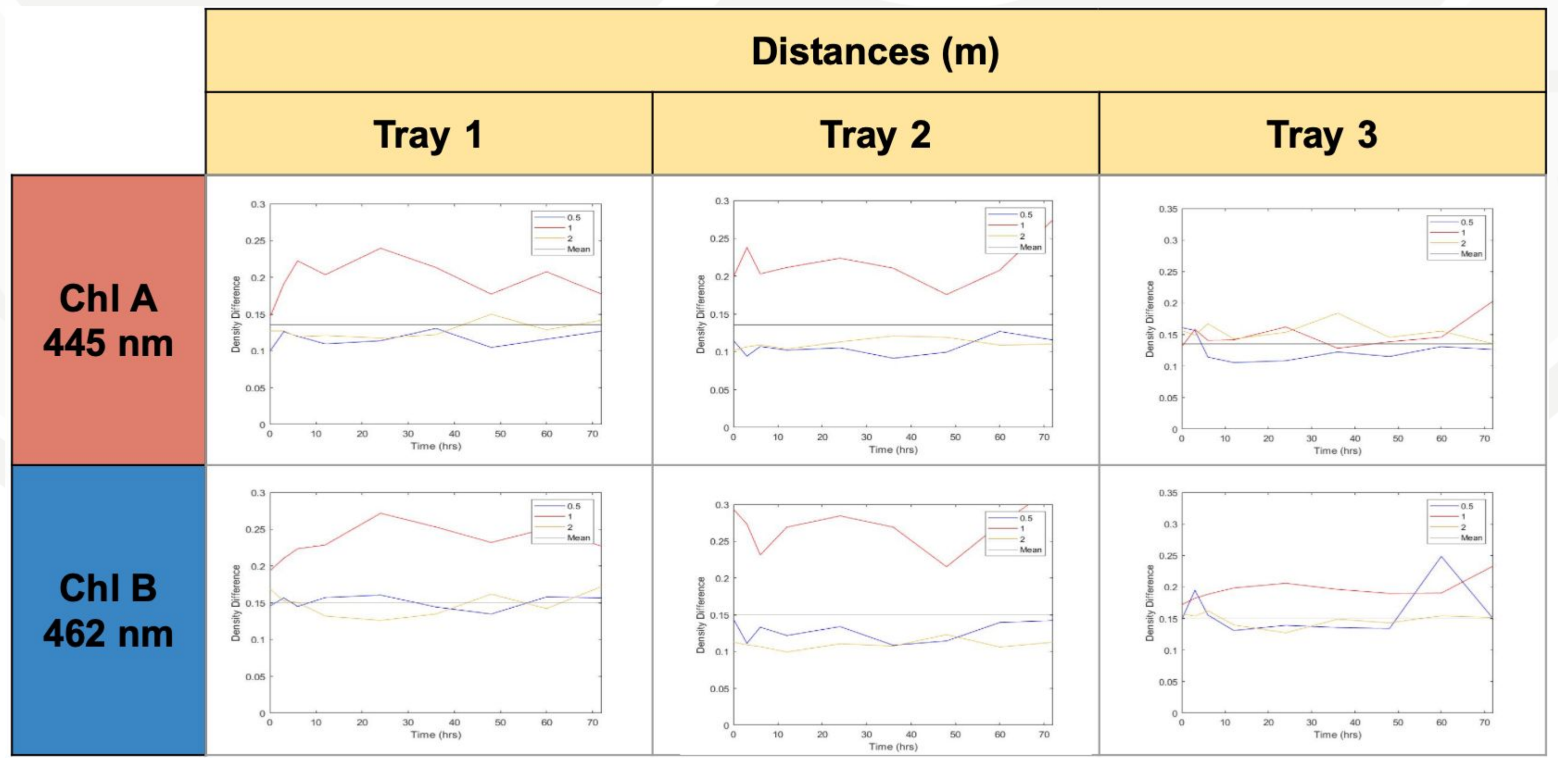
Technical Approach

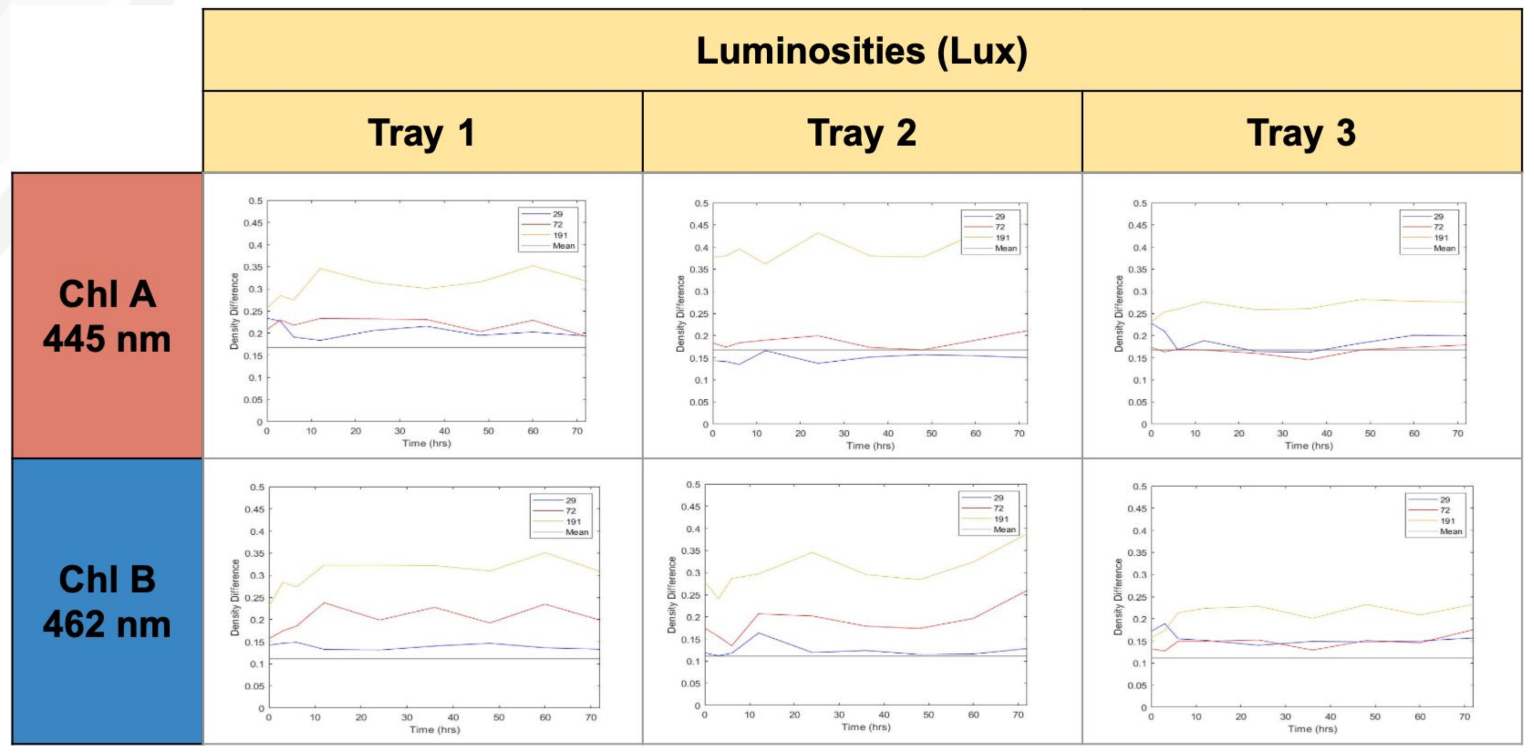
Azolla filiculoides, an aquaponic waterfern, was used as the study plant. It was cultivated in the laboratory under greenhouse conditions prior to and during treatment. For the experiments, azolla was separated into 3 (22-5/8" L x 15-1/2" W x 2" D) trays with 2500 mL deionized water each and increasing concentrations of Lead (II) Chloride (0.1 nmol/cm^3, 1 nmol/cm^3, and 10 nmol/cm^3). Initial imaging of these trays to produce baseline control images were performed 12 to 24 hours before dosage and responses to metal were captured at hours 0, 3, 6, 12, 24, 36, 48, and 72 after metal addition to the solution. The project focused on imaging each tray from various laser and camera heights (0.5 m, 1 m, and 2 m) and light conditions (29 Lux, 72 Lux, and 191 Lux) using lasers with wavelengths of 445 nm and 462 nm. LIF responses were captured as color images using a CMOS camera, from which pixels were extracted and analyzed for RGB color distributions. Red color histogram differences between control images and images of metal-dosed plants were calculated to show plant physiological changes as a response to exposure to lead.



Results

Differences in red color histograms between control and metal-dosed plants at distances 0.5, 1 and 2 m show that density differences are greatest at the 1 meter distance for samples with 0.1 nmol/cm^3 and 1 nmol/cm^3 for chlorophyll a and b specific lasers. All trays experimented with the 191 Lux luminosity had the highest density differences although decreased as Pb concentration increased.





MTV Impact

This work was performed under supervision of Dr. Henrietta Dulai. MTV supports undergraduate student Haley Currier and graduate student Kelly Truax who, in collaboration with the national laboratories developed and designed this project. Savannah River National Laboratory aided in the application of bioremediation potential of this work in regards to the removal of nuclear waste and heavy metal contamination. Lawrence Livermore National Laboratory provided the knowledge on laser system and calibration design for the chlorophyll specific experiments. MTV continues to guide future collaborations with national laboratories to build on the efficiency and improvement of this detection method in environmental monitoring.

Conclusion & Next Steps

Testing light conditions and distances at which the laser systems can be used further improves our understanding of the limitations of this technique, especially when planning for in-situ remote sensing design. Future experiments will continue testing these conditions and improve the system to be less dependent on variables, for example by applying laser bypass filters that cut out unwanted wavelengths of light that target chlorophyll a and b absorption peaks. More tests with different plant types will also help determine the best candidate for bioremediation use in a field setting.

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