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Determination of *Microcystis aeruginosa* Concentration Using Two Discrete Wavelengths

Harmful algal blooms (HABs), specifically Microcystis aeruginosa (MA), present a serious global problem to bodies of water. HABs are the rapid growth of toxic algae species in a waterway. These algae species are known to cause irritation, nausea, and vomiting in humans, and even more severe side effects in smaller organisms. Climate change and human development have caused these harmful blooms to become more prevalent in recent years. Current commercial and academic algae detection methods were researched and found to be highly restrictive or expensive. This creates the need for a monitoring device that fills this niche, which the team attempted to do. Regarding the detection of MA, the peak spectral absorbances were determined to be at wavelengths of 430 nm and 680 nm. The handheld harmful algae monitoring device directs these specific wavelengths of light matching the peak absorptions of MA through a sample. The relative intensity of light after passing through the sample is measured and used to determine the presence and concentration of MA. This detection method is low cost, is portable, and will provide efficient and precise results with the hope of enabling a variety of users on a large scale. With proper calibration and more research, the handheld harmful algae monitoring device has the potential of being highly accurate and capable of testing nonpure samples.
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1 Introduction

Harmful algal blooms (HABs) pose an enormous threat to waterways and ecosystems [1,2]. Most algae are beneficial to the ecosystem they reside in as they provide food for marine life and produce oxygen, but some algae are considered harmful. Harmful algae are species of algae that produce toxins that can poison marine life and grow at uncontrolled rates [3]. Once algae in an area are producing toxins or growing uncontrollably, they are considered harmful algal blooms. Current methods of detecting algae are costly and cannot provide rapid detection. The team attempted to design a handheld, low-cost, and efficient device capable of detecting the presence and concentration of harmful algae in a pure sample.

The first step to detecting HABs was to identify a common species of algae. According to a study over the span of 5 years, the most prevalent harmful algae species in a river were *Aphanizomenon flos-aquae*, *Microcystis aeruginosa* (MA), and *Anabaena spiroides* [4]. MA is specifically found in freshwater and brackish ecosystems and is a very common cyanobacteria. The toxins produced from these types of harmful algae are concentrated in the shellfish that humans eat and lead to 50,000–500,000 illnesses per year [5,6]. Based on this research, as well as the advice from several scientists at the Woods Hole Oceanographic Institute (WHOI), MA was chosen as the species of interest for this project.

The team designed a device capable of detecting the presence and concentration of MA in a pure sample. The goal was to present a functional prototype that utilized the visible light and sensors to analyze a sample of harmful algae. The device would then display the presence and concentration of MA to researchers. The team's prototype is 3D printed and designed with intent for mass manufacturing through plastic injection molding. Similarly, the team's prototype uses an Arduino Nano to process and display data.

The team succeeded in creating a final product that is cheaper, smaller, and quicker than existing devices. This will allow for more lab personnel and environmental agencies to obtain the product and conduct rapid tests. Ultimately, the handheld harmful algae monitoring device will provide confidence in its results for the users and serve as a precursor for a more advanced product that can test for different species of harmful algae in nonpure samples.

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2 Background Review

It is important to understand HABs and their impact, as well as current standards, principles and concepts, literature, and products employed in the field. Patents are another important aspect of research; however, no patents were found that were relevant to the team's design. HABs have recently grown in severity and have a multitude of detrimental effects on animals and society [7]. There are two main standards for determining the presence of harmful algae: cyanobacterial cell counts and toxicity levels. In addition, the team researched several other detection methods that include optical biosensing, spectroscopy, unmanned aerial vehicle (UAV) detection, LiDAR detection, the BenthoTorch, and the AlgaeTorch. Since the target audience of the HAB monitoring device is agencies like the Environmental Protection Agency (EPA) and lab personnel that test algae, it is essential to design a product that is low cost, portable, efficient, and accurate. The background research shared later helped to ensure that all the criteria are met and optimized in the team's design.

2.1 Harmful Algal Blooms. Harmful algal blooms are a serious problem in global water supplies and generate ecological instability. In recent years, HABs have rapidly expanded in size, frequency, and severity due to human overpopulation and excessive contamination of the environment, see Figs. 1 and 2.

HABs have many negative effects on health, ecosystems, and socioeconomics. For instance, the toxins produced by harmful algae can potentially poison and kill marine life, animals, and humans [8,9]. This is demonstrated in Fig. 3.

Harmful algal blooms also disrupt ecological structures and functions, drinking reserves, industries, tourism, and much more [8,11]. In other words, harmful algal blooms cause regional industries to struggle, maintenance and healthcare costs to increase, and environmental destruction. Eventually, HABs can cause irreparable damage leaving water reserves as dead zones and unsuitable for life [12,13].

It is clear that HABs are instigated by human activity and are disastrous for the environment and society. This creates the need for an accessible, portable, cost-effective, accurate, and efficient harmful algae monitoring device. The device will allow agencies and lab personnel to reduce costs, increase testing efficiency, and work in a safe and confident manner. With this, more HAB data can be obtained in attempts to mitigate their negative impacts.

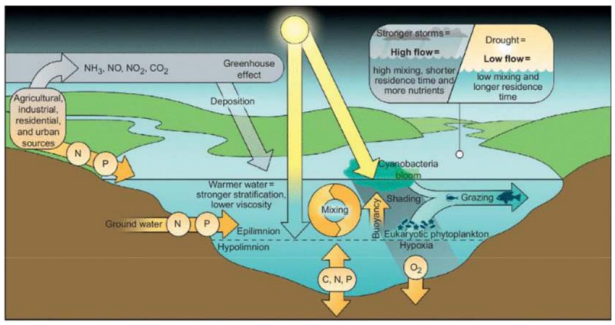

Fig. 1 Illustration of factors that lead to HAB accumulation [8]

Fig. 2 Lake Eerie experiencing a HAB [8]

Fig. 3 Image of aquaculture devastation due to HABs [10]

2.2 Standards. Due to the heightened presence of HABs, many organizations around the world are monitoring harmful algal growth and conducting water quality tests. The two principal standards for detecting HABs are total cyanobacterial cell counts and toxicity levels.

The easier detection standard is total cell counts, in which there are three cyanobacterial bloom risk levels: low, medium, and high. The World Health Organization (WHO) low risk level ranges from 0 to 20,000 cells/mL, the medium risk level ranges from 20,000 to 70,000 cells/mL, and the advisory level is greater than 70,000 cells/mL [14]. The handheld algae monitoring device will adhere to these concentration levels, which are demonstrated in Fig. 4.

The more complicated detection standard is toxicity levels. There are several different types of toxins emitted from HABs, the most common being microcystin. The WHO suggests drinking water microcystin limits of 1 part per billion (ppb) and recreational water limits of 20 ppb [15]. Furthermore, there is a correlation between cell counts and toxicity levels; however, the degree of their relationship is still unknown. Cell counts of 20,000 cells/mL, 70,000 cells/mL, and 100,000 cells/mL have approximated toxin concentrations of 2–4 ppb, 14 ppb, and 20 ppb [15]. Therefore, the team's algae monitoring device could correlate the calculated algae concentration with a level of toxicity and output that to the user.

2.3 Principles and Concepts. The most crucial principles and concepts when detecting HABs were found to be optical biosensing with an emphasis on spectrometry. This research was used to design

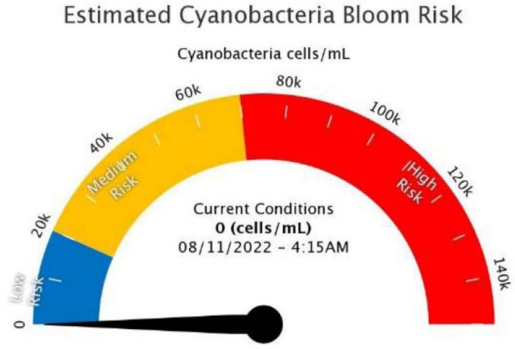

Fig. 4 Estimated cyanobacterial bloom risk meter [14]

the method with which the harmful algae monitoring device detects the presence of algae and returns concentration data to the user.

Currently, there are several different techniques used to detect HABs. In particular, optical biosensing is a method that delivers high specificity, fast, easy to use, and on-site detection [16]. These qualities most closely adhere to the requirements of the handheld harmful algae monitoring device. Thus, more research was conducted regarding optical biosensing. Within this technique, the team focused on UV and visible spectroscopy. Spectroscopy works by obtaining the absorbance spectra of a compound in a solution. Absorbance can be found using the following equation:

$$A = \log\left(\frac{l_0}{l}\right) = \log\left(\frac{V_{\text{Sample}} - V_{\text{zero}}}{V_{\text{Solvent}} - V_{\text{zero}}}\right) \quad (1)$$

where A is the absorbance, l_0 is the initial intensity of the light source, and l is the intensity of the light that was able to pass through the sample [17]. Furthermore, absorbance and concentration are related to each other through the following equation:

$$A = \varepsilon L c \quad (2)$$

where e is the molar absorptivity, L is the path length of the cuvette or sample holder, and c is the concentration of the solution [17]. Spectroscopy tests are usually setup with a light source, the sample, and a detector as shown in Fig. 5.

By varying the wavelength, a plot of the absorbance as a function of wavelength can be generated. This graph reveals the peaks in absorbance, which can be correlated to the presence of specific materials, see Fig. 6.

When designing the algae monitoring device, it was essential to understand all principles and concepts within that system to ensure its effectiveness. Employing optical biosensing in the form of UV and visible spectrometry in the product reduced the cost and size constraint, increasing its accessibility and portability. In addition, spectrometry allowed for the device to accurately detect algae in a range of concentrations. The harmful algae monitoring device design incorporated an internal test setup like Fig. 5. Although the actual device will not output an absorption spectrum, the principle is helpful when studying algae.

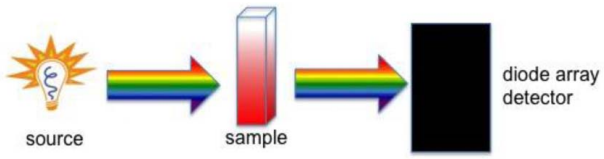

Fig. 5 Simultaneous spectroscopy setup [18]

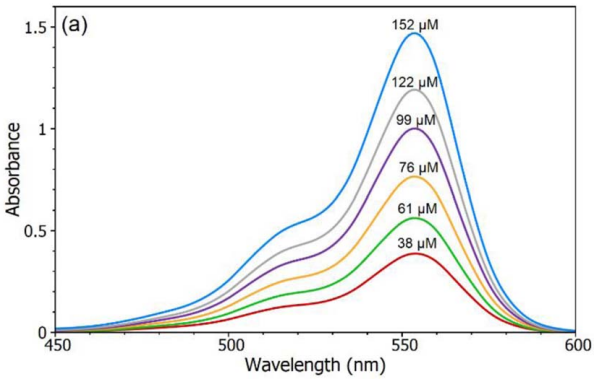

Fig. 6 Example plot demonstrating the absorbance of a material at different wavelengths [19]

2.4 Literature. HAB detection and analysis experiments are occurring globally from the United States to Korea and Japan. In recent years, the EPA, and Massachusetts Water Resources Authority (MWRA) installed a live monitoring buoy into a river, and water samples were manually collected to compare the total cyanobacterial cell count data [14]. Manual cell counts were completed in a laboratory using a Sedgewick–Rafter counting slide, which measures the exact number of particles in a volume of fluid [4]. The study found that HABs occurred almost every year, and the physical and live data comparison created a foundation for forecasts to be made, see Fig. 7.

Unfortunately, this type of research is restrictive in a sense that it is time consuming and costly. Each sample takes time to collect and prepare. Additionally, the required analysis equipment is prohibitively large and expensive.

Similarly, researchers in Korea and Hungary have experimented with monitoring HABs through UAVs [20]. In the studies, the UAVs were used to take photographs of various water bodies. Then, the images were analyzed using color-sensing technology. The researchers used the hue, saturation, and lightness model of color detection, in which they successfully detected blue-green algae 80% of the time [21]. This detection method is limited because UAVs are costly, they require trained operators, and the HAB must be large enough to be caught on camera from a distance.

In Japan, researchers developed a method to detect HABs through LiDAR. This technique consisted of shining a UV laser with a wavelength of 355 nm, pulse width of 6 ns, and a frequency of 10 Hz through algae samples to reveal their florescent spectra. Each species of algae demonstrated a unique peak in the intensity versus wavelength graph, allowing them to distinguish between algae species [22]. This is demonstrated in Fig. 8.

This type of research is restrictive because the equipment is large, and the required preparations are time consuming. Moreover, the technology includes the use of a strong laser, which is inaccessible to most.

In the end, numerous organizations were found to be monitoring the presence of HABs. However, current detection methods are too large, costly, or inefficient. This creates the need for an accessible, portable, cost-effective, accurate, and efficient harmful algae monitoring device. The team's proposed product will attempt to meet all these requirements. Moreover, the concepts of using a Sedgewick–Rafter counting slide and graphing material properties as functions of wavelength proved to be important tools for examining algae.

2.5 Products. There are a few algae detection products currently on the market; however, each is limited in its accessibility or application. For instance, there are some cheaper options available such as algae test strips, and then there are immensely more expensive options like UAV detection. Often, available detection products are on opposite sides of the spectrum and are lacking with respect to the user's needs.

Two commercialized products that are the most similar to the team's algae monitoring device design are the BenthoTorch and the AlgaeTorch made by BBE Moldaekne. The BenthoTorch specializes in detecting benthic algae by exciting internal pigments [23,24], while the AlgaeTorch is made to detect cyanobacteria by exciting chlorophyll pigments [25]. Although both products are smaller and more portable, they are extremely expensive and inaccessible. For example, they are not easily obtainable in traditional stores or via the Internet.

Some noncommercialized products used to detect HABs include UAV and LiDAR detection. These devices are large and expensive technologies that are far more complicated to implement. Furthermore, there are several limitations to these products. Regarding UAV detection, the technique only works after the HAB has already occurred and covers a large area. Therefore, low concentrations of algae would not be detected. Also, LiDAR does not work on all types of algae and is very difficult to use.

In summary, there are some existing algae detection products, but they all have design flaws. Many devices are too large, costly, or

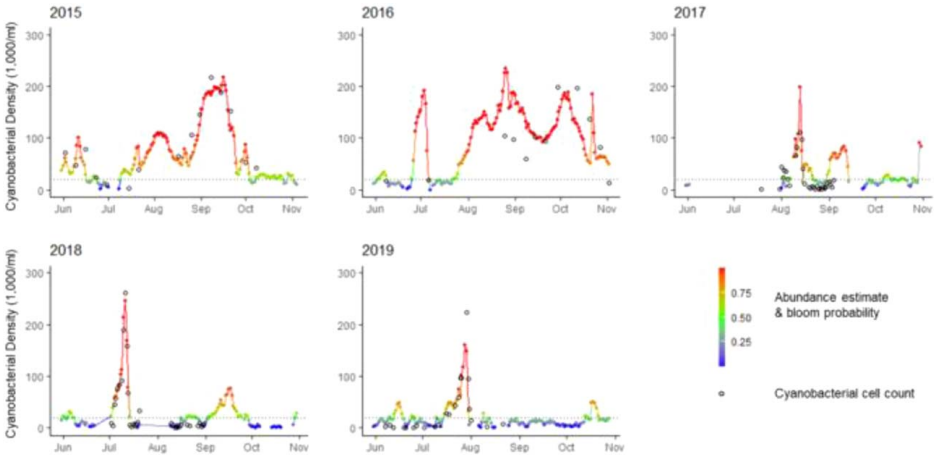

Fig. 7 Comparison plot of total cyanobacterial cell counts and live buoy data from 2015 to 2019 [4]

complicated to operate. The team's handheld harmful algae monitoring device will be designed to address these issues, in which it will be portable, inexpensive, and simple to use. This will allow the product to be more accessible to agencies and lab personnel. As a result, more experimentation can occur with our device to correlate algae concentrations with various physical phenomena in attempts to characterize HABs and mitigate their environmental impacts.

2.6 Research Summary. There are several elements that were considered when designing the handheld harmful algae monitoring device to guarantee its effectiveness and conformity with the team's needs. This includes learning about HABs, current standards, principles and concepts, literature, and existing products. Although patents were thoroughly researched, none were found that guided or hindered the team's design. Along with the growth of HABs and their disruptive nature, more agencies around the world are conducting research regarding their detection and analysis. These tests follow standards regarding the intensity of the bloom and amount of toxins present. The harmful algae monitoring device will utilize UV and visible spectroscopy to detect the presence and concentration of specific algae species, such as MA. The most similar products that exist in the market today are the BenthoTorch and the AlgaeTorch, but their high costs restrict their accessibility. Other solutions exist such as UAV and LiDAR detection, but they too are costly, large, complicated, and inconsistent. Ultimately, sources across research topics align in the sense that there are existing solutions to detect the increased presence of harmful algal blooms, but the solutions are often lacking in one way or another. The team's handheld algae monitoring device will distinguish itself from other products through its portability, low cost, accuracy, and efficiency. This will provide an appealing and innovative solution to the market.

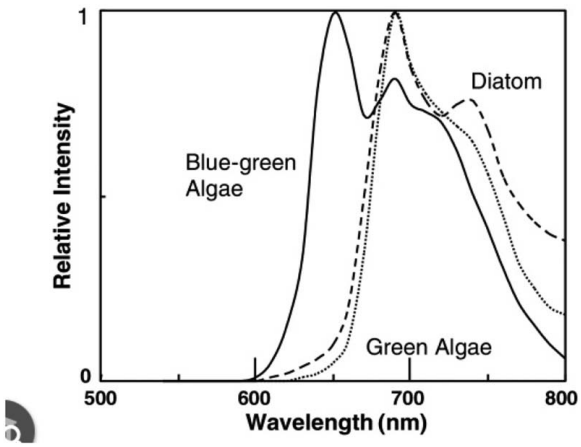

Fig. 8 Intensity graph used to find unique peaks for the detection of algae [22]

3 Analysis

3.1 Experimental Process. A critical component to the design process was the team's preliminary research. The absorption spectrum of MA had to be identified before any components could be purchased or any product design could be undertaken. It was also necessary to determine the absorption spectrum of other common algae species. Two experiments were designed to gather these two data sets.

Before completing these experiments, the Woods Hole Institute was contacted to provide samples of a multitude of algae species, a mineral water growing medium, test tubes, as well as Sedgewick–Rafter counting slides. Additionally, the team purchased a handful of other materials including a micropipette and a cuvette specialized for spectrophotometry.

The first experiment focused on the absorption spectrum of MA and how that spectrum varied with changes in concentration. To test this, an experimental procedure was drawn up and executed. First, a baseline absorption spectrum of our cuvette and the pure mineral water solution was found. The cuvette was then emptied and filled with our sample of MA, which was dissolved in mineral water. This sample was tested in the spectrophotometer four times per sample and then emptied out into our counting slide and placed under the microscope. It was found to be necessary to leave the sample undisturbed for 5 min before attempting to observe it under a microscope. This time allowed all the algae cells to fall to the bottom of the slide and appear clearly under the microscope. Four pictures from different areas of the sample were then taken, which were later used to calculate the algae cells per milliliter of mineral water and its error. This process was repeated five more times, and in each subsequent test, the sample was diluted with more mineral water. The experiment results are visible in Fig. 9.

The lines in Fig. 9 show the different absorption spectrum of MA with varying concentrations. This graph revealed two absorption peaks, which the group theorized could be used to consistently detect MA, one peak at 680 nm and the other at 430 nm. From these data, it was then helpful to pull out the trends at the two peak wavelengths of interest, and plot them against the concentration calculations found with the microscope. The data are presented in Fig. 10.

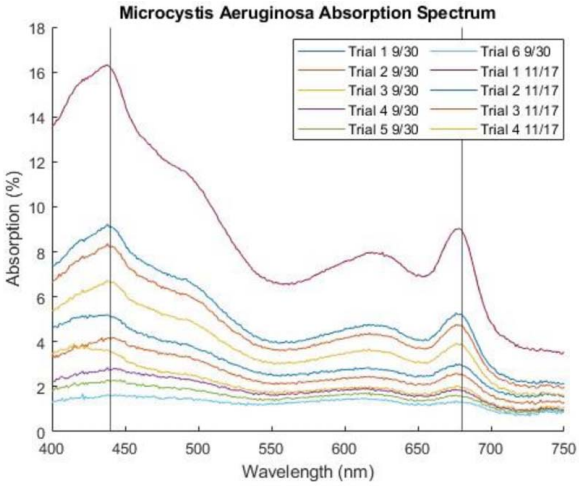

Fig. 9 MA absorption spectrum at varying concentration

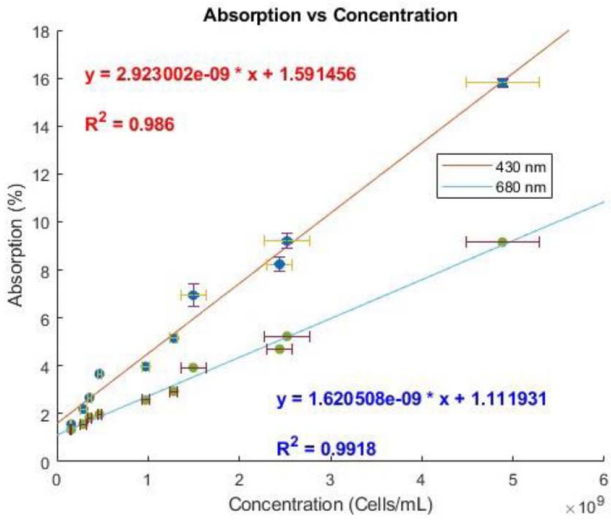

Fig. 10 Regression line fitting of concentration versus absorption plot for MA with error bars

This plot revealed a helpful trend in concentration and absorption, as concentration increased, so did the percent absorption of the sample. The R^2 value of 0.986 and 0.9918 for the absorption at 430 nm and 680 nm, respectively, gives strong confidence that the relationship between absorption and concentration is linear.

The second experiment was designed to acquire data on the absorption spectrum of a multitude of different algae species. It was not necessary to vary concentration in this experiment as the shape of the absorption spectrum was the only data needed. To achieve this, the cuvette was filled with one of the many species of algae acquired from Woods Hole, tested in the spectrophotometer, and then emptied out. This process was repeated for all the unique species available. Results are shown in Fig. 11.

MA is again visible in red, and when compared to the other algae species on hand, it was clear that the wavelengths initially thought to be promising candidates for detection, 680 and 430 nm, and did in fact have a distinct relationship when compared to the other algae species. This relationship could be used to distinguish MA from a multitude of other algae species.

3.2 Design Criteria. The experimental process revealed multiple criteria needed in the product design. The two main discoveries were that two LEDs could be used to determine the concentration of MA, one which emits light at 430 nm and the other which emits light at 680 nm. Additionally, a sensor capable of detecting light at both wavelengths would be necessary. During our experimental process, it was also learned that spectrophotometry has a standard 6 deg angle that absorption is calculated at, and this angle would need to be replicated in the design as well.

A few standard requirements were also agreed upon. The design was expected to include an LCD screen that would display results and be portable. A rechargeable 12-V battery pack was decided upon to eliminate the need to plug the sensor into a wall outlet. Additionally, a microprocessor was needed to interpret the collected data, and the Arduino Nano was chosen for this role. It was also imperative to minimize light pollution in the testing chamber as this could skew results.

3.3 Design Process. From the team's research, it is known that the designs needed to have a user-friendly interface. Specifically, the cuvette needs to be accessible, intuitive, and easy for the user to insert and remove. Steps also need to be taken to simulate the inside of a spectrophotometer such as minimizing ambient light reaching the inside of the device. The design also needs to be

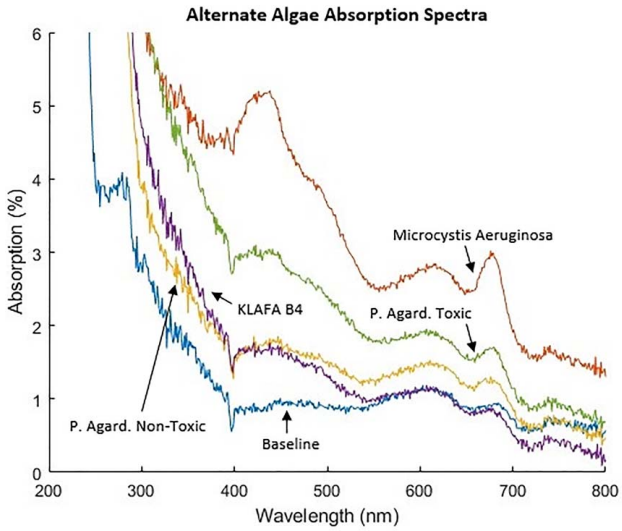

Fig. 11 Plot of the absorptions of different species of algae at varying wavelengths

small enough to be handheld, but big enough to accommodate wiring and assembly of all components. The team's design process involved initial sketches, SOLIDWORKS modeling, rapid prototyping of the model, and finally assembly and testing.

3.4 Final Design. The final design is shown in Fig. 12. This design has all mounting holes modeled with screw inserts and M2 or M3 screws. It has guide rails along the top and bottom of the cuvette insertion. An Arduino Nano microprocessor is included in this design with a cutout to allow for exporting data or uploading code to the device. This can be seen in the bottom left of Fig. 12. This iteration addresses all issues found in previous rounds of design.

This iteration also adds a separate subassembly, shown in Fig. 13, the buttons used to operate the device. The subassembly is mounted to the small half of the device, which allows the black pieces to "float" and then activate the fixed buttons below them when the user presses these components.

3.4.1 Execution of Prototype of Final Design. The final design, shown in Fig. 14, is printed on the onyx plastic material. This black material was expected to mitigate the reflection of any ambient light. This idea was validated when the sensor output was almost 0 V with no LEDs activated.

To ensure the mechanics of the device worked properly, the devices had to be wired correctly so that each component received the proper voltage. The wiring of the device can be seen in Fig. 15.

The screen was wired using a ribbon cable and a breakout board. All components needed to be wired to the Arduino Nano, as it acted as the brain of the device. All inputs and outputs are controlled by the Arduino, including the buttons, display, battery, detector, and LEDs. To ensure all connections were secure and shielded, heat shrink was used to cover each wire interface. Additionally, most wire colorings were kept track of, specifically red wires used for power, and black wires used for grounding. Other colors were used for input or output connections to/from the Arduino. This final design incorporates all the components shown in Table 1.

Fig. 12 Final design of handheld harmful algae monitoring device

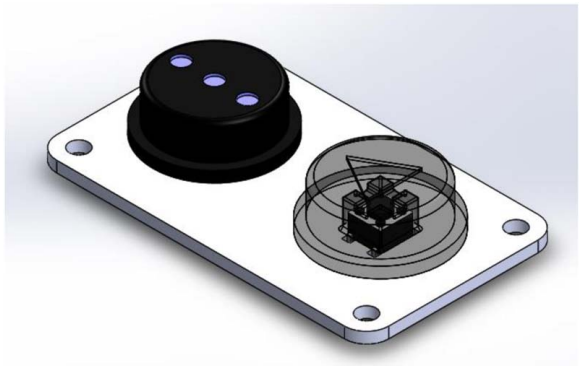

Fig. 13 Button subassembly of final design

Fig. 14 3D printed final design of handheld harmful algae monitoring device

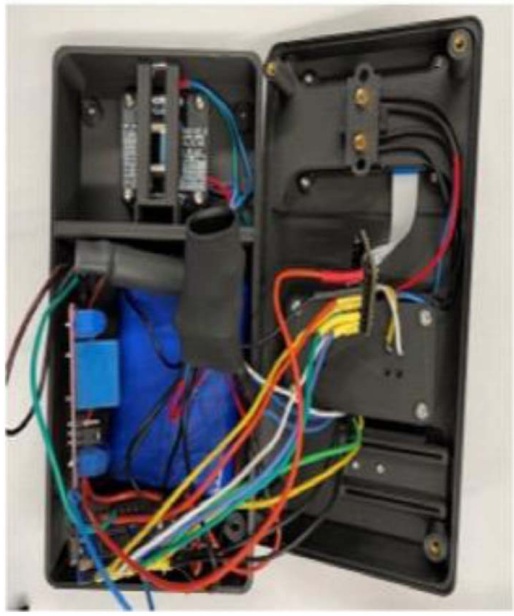

Fig. 15 Internal wiring and setup of final design

3.5 Calibration. After the final design was assembled, it was calibrated by using the spectrometer as the known measurement, and the device with two LEDs as the experimental measurement. The calibration procedure for absorption was conducted for both the 430 nm and 680 nm wavelengths and is shown in Figs. 16 and 17.

The respective R^2 values of 0.9979 and 0.9996 show a strong linear relationship between the spectrometer values of absorption and the device's calculated value of absorption using Eq. (1).

3.6 Applications. The sensor in the device first measures the ambient light voltage followed by the voltage when the LED lights are individually activated and passed through the solvent. The device then similarly measures the voltage received when the individual lights pass through an MA sample. These values are

Table 1 Bill of materials for final design of handheld harmful algae monitoring device

Item no.	Part number	Description	Quantity	Total cost
1	Big Half	Bottom half of shell (Onyx)	1	$19.19
2	Small Half	Top half of shell (Onyx)	1	$13.69
3	YB1203000	Rechargeable 12 V battery	1	$27.39
4	PDAPC2	Si Switchable Gain Detector on PCB, 320–1100 nm	1	$181.62
5	Cuvette	2 mm cuvette, 0.7 ml	1	$35.00
6	LED Assy.	430 nm LED, 680 nm LED	1	$24.13
7	5394	Adafruit 1.9 in. 320 × 170 Color IPS TFT Display - ST7789	1	$17.50
8	Button Mounting	Tactile Switches	1	$2.48
9	B07G99NNXL	Arduino Nano	1	$8.33
11	Hardware	Helical Inserts and Screws	1	$9.84
	–	–		$339.17

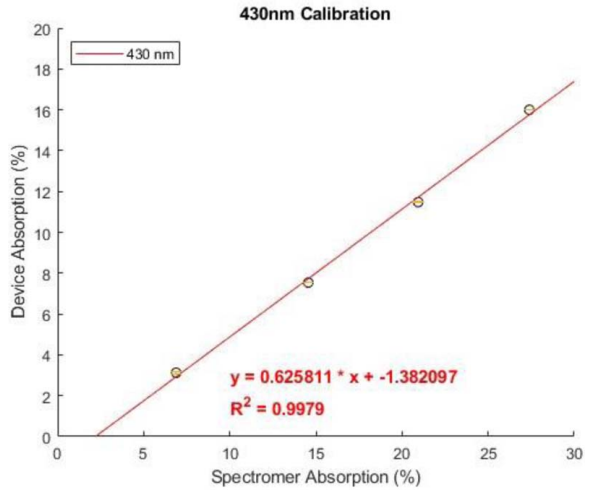

Fig. 16 Calibration curve for final product at 430 nm

used to calculate the absorption using Eq. (1) and are then calibrated using the trend lines in Figs. 12 and 13. Using the calibrated values of absorption, the concentration of *Microcystis aeruginosa* can be determined with the trend lines shown in Fig. 6. The concentration is individually determined using each discrete wavelength and is then compared to each other to validate the result. If the concentration values are drastically different from each other, then it can be concluded that the sample is not MA.

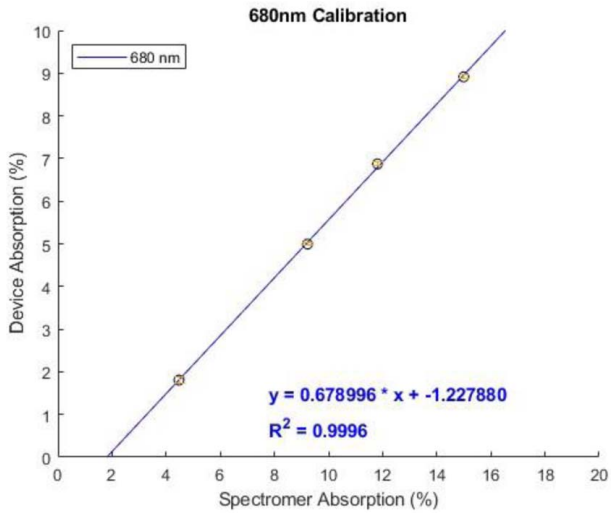

Fig. 17 Calibration curve for final product at 680 nm

4 Discussion and Conclusion

The team found great success in developing a method of simplifying spectrometry to determine the absorption of an algae sample. The team discovered that even though a spectrometer can be used to pass the entire visible light spectrum through an algae sample and obtain its entire absorption spectra, only peaks of the absorption spectra are of interest when attempting to determine the concentration of a sample. This allowed the team to use only two wavelengths of light instead of 800 discrete wavelengths. Strong evidence of the positive linear relationship was discovered between the absorption and concentration of an algae sample at these wavelengths. The strongly linear relationship between the calculated absorption of the team's device and the spectrometer gave confidence in this method. When measuring the absorption of an algae sample, the team's device had a precision of 2%, further validating the method.

Using this absorption, the device can calculate and display the concentration of the sample in 10 s. This method of determining concentration is significantly more efficient than manual cell counting techniques under a microscope, which can take multiple hours. By using this device, any person can quickly test the concentration of multiple algae samples and determine if further laboratory testing needs to be conducted.

The limitations of this device stem from the accuracy of the team's cell counting method. The team encountered many challenges with cell counting such as not being able to determine which cells are alive, not having a consistent solution of the sample across the counting slide, and cell counting software not counting certain cells. These variables in the cell counting procedure may have led to inaccurate concentrations shown in Fig. 6. Since the team was consistent in how cell counting was conducted, the linear trend lines shown in Fig. 6 are still strongly correlated but may not be exactly accurate. Future work would involve stronger cell counting techniques such that the accuracy of these trendlines could be improved. An additional limitation of this method is that only a pure sample of algae can be tested. If there are any contaminants in the solvent, the absorption spectra would be altered, and the trendlines would no longer be valid. Further research would need to be conducted to see how contaminants alter these trendlines.

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Conflict of Interest

There are no conflicts of interest. This article does not include research in which human participants were involved. Informed consent not applicable. This article does not include any research in which animal participants were involved.

Data Availability Statement

The datasets generated and supporting the findings of this article are obtainable from the corresponding author upon reasonable request.

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