ANALYZING A LOW-COST, CUSTOM-BUILT UV-VIS SPECTROSCOPY SETUP WITH MACHINE LEARNING METHODS TO PREDICT NITRATE CONCENTRATIONS IN HYDROPONIC NUTRIENT SOLUTIONS

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Purpose

This research project stemmed from a larger, ongoing project for developing field-deployable water sensors using a low-cost, custom built spectroscopy setup called the GatorSpec. Originally a research assistant for Dr. Aditya Singh, then for Dr. Eban Bean in the Urban Water Resources Lab in the Agricultural and Biological Engineering (ABE) Department at the University of Florida (UF), the author received an undergraduate research fund to perform an independent research project. Since the GatorSpec had not been validated yet, the aim of this research project was to validate GatorSpec results by comparing them with that of a state-of-the-art, off-the-shelf spectrophotometer, the ThermoFisher NanoDrop™ 2000C.

The GatorSpec was built by Barrett Carter, a PhD candidate in the ABE Department at UF. Barrett also created the recipes for the synthetic hydroponic nutrient solutions. The author created the solutions from the recipes, analyzed them on the GatorSpec and NanoDrop2000C, developed the model to predict concentrations of nitrate in the samples, and performed the statistical analyses on the data.

Acknowledgements

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Abstract

Gathering water quality information is important for understanding and managing of water resource systems. For instance, hydroponics is an important water-focused system for crop production, and the correct management of nutrients in a hydroponic system is necessary for producing healthy crops efficiently and sustainably. However, it is relatively expensive and tedious to collect information on water quality using conventional methods. UV-Vis spectroscopy is a highly established laboratory technique used for water quality analyses. A main nutrient of concern in hydroponic systems is nitrate (NO3-N), which can be measured with UV-Vis spectroscopy. The GatorSpec UV-Vis spectroscopy setup was built by a PhD candidate in the Agricultural and Biological Engineering Department at the University of Florida as a low-cost method for gathering environmental water quality data. The goal of this research was to validate this spectroscopy setup. This was done by gathering UV-Vis absorbance data on 46 synthetically made hydroponic nutrient solutions using the GatorSpec, as well as a commonly used commercial spectroscopy setup, the NanoDrop2000C. Further, this data was analyzed with two statistical methods, principal component analysis (PCA) and partial least squares regression (PLS). PCA results showed that when the same analysis is performed on both sets of data, the outcomes are very similar, indicating the absorbance data from the GatorSpec provides similar information to that of the NanoDrop2000C. The PLS results revealed that for the diluted samples, the models derived from both machines created was very good at predicting nitrate concentration. With these outcomes, it can be concluded that the GatorSpec has a comparable performance to that of the NanoDrop2000C and functions well as a UV-Vis spectrophotometer. This low-cost setup can be used to determine nutrient concentrations in hydroponic nutrient solutions.

Introduction

UV-Vis spectroscopy is "one of the most well-established techniques for chemical identification and quantification" (Silva et al., 2021). UV-Vis spectrophotometers measure the light absorbed, reflected, or transmitted in a sample at wavelengths across the UV to visible range (Vitha, 2018). This range is normally 190 nanometers to about 700 nanometers. With the output spectrum from the machine, chemical and physical properties of substances within the sample can be identified. With the absorbance values obtained from UV-Vis spectrophotometers, the concentration of a particular molecule in the sample can be determined (Vitha, 2018). Current methods of gathering UV-Vis spectroscopy data and water quality data are rather expensive and tedious (Huebsch, 2015). Furthermore, when solutions are not pure and there is interference from other compounds, it becomes increasingly difficult to obtain accurate measurements (Silva et al., 2021).

The quality of water in the United States and throughout the world is being impaired as human population increases and the demand for food from agriculture increases (Sambo et al., 2019). While the climate crisis grows and more problems arise in agricultural settings due to pathogens and pollution, it is becoming less feasible to grow crops using conventional methods (Sambo et al., 2019).

One example of a system that can battle this problem and grow plants in a setting that exploits less environmental resources is hydroponics. Hydroponics is a system that utilizes a water-based, nutrient-rich solution to grow plants. This nutrient solution is an aqueous solution that contains many soluble salts and inorganic ions that are essential for plants to grow (Trejo-Téllez & Gomez-Merino, 2012). Currently, there are 17 essential nutrients for most plants grown in hydroponics: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, copper, zinc, manganese, molybdenum, boron, chlorine and nickel (Salisbury & Ross, 1994). All of the most basic hydroponic nutrient solutions contain nitrate (Trejo-Téllez & Gomez-Merino, 2012).

One of the current disadvantages of a hydroponics system is that it requires constant monitoring and quantification of nutrients (Silva et al., 2021). The plants grown in hydroponics require specific levels of various nutrients to help them grow and reproduce. There are specific levels of ion activity that plants require to stay healthy, and if these levels go higher or lower than the accepted range, the nutrient solution could become toxic or deficient to the plants (Bamsey et al., 2012). The chemistry of the solution in hydroponics is extremely important for ensuring proper nutrient uptake from the plants (Sambo et al., 2019).

Due to the sensitive nature of hydroponics, monitoring and managing the level of nutrients in a hydroponics system is essential to its success and ability to produce the plants desired. Many current hydroponic plant growers solely rely on visually detecting deficiency or toxicity from problematic nutrient levels (Bamsey et al., 2012). One of the main chemical parameters that helps assess the quality of water is the presence of nitrogen compounds (Salivon et al., 2018). Nitrate levels have a large effect on plant growth in hydroponic systems. Nitrate is measured at wavelengths within the range of 210 to 240 nanometers in the UV-Vis spectrum (Salivon et al., 2018).

The GatorSpec, an open-source, custom-built, low-cost benchtop spectroscopy setup was created by J. Barrett Carter, a PhD candidate in the Agricultural and Biological Engineering Department at the University of Florida, for an ongoing project to form the technological basis for developing field-deployable water sensors. The goal of creating the GatorSpec is to be able to accurately collect water quality data at a high frequency and a low cost. Current UV-Vis spectroscopy technologies cost upwards of $20,000, while the GatorSpec setup costs roughly $2,500 to construct. The GatorSpec's ability to collect accurate data has not been validated against a standard instrument. The NanoDrop2000C is a highly accurate, full-spectrum spectrophotometer that is commonly used in laboratories.

The objective of this study was to validate a low-cost, custom-built, open-source spectroscopy setup as a water quality sensor by comparing its ability to collect water quality data in hydroponic nutrient solutions to that of a state-of-the-art, off-the-shelf, standard laboratory spectrophotometer. It was hypothesized that the function of the two machines would not be significantly different based on the information produced by each and its relationship with the chemical composition of the samples. If the

GatorSpec could be validated, it could be used to cheaply and effectively advance monitoring and management in hydroponic systems, as well as other water systems.

Materials and Methods

The goal of this project was to validate a low-cost, custom-built, open-source spectroscopy setup, the GatorSpec, by comparing it to a state-of-the-art, off-the-shelf, standard laboratory spectrophotometer, the NanoDrop. More specifically, the machines were compared based on the results of statistical analyses performed on the data produced by each machine and by comparing their ability to predict the nitrate concentrations in synthetic hydroponic nutrient solutions. Due to the interference of other concentrated compounds when analyzing hydroponic nutrient solutions with spectroscopy, machine learning methods were used to approximate complex mathematical relationships and derive statistics to provide the most relevant information to compare the two machines (Silva et al., 2021).

Synthetic Hydroponic Nutrient Solutions

Forty-six synthetic hydroponic nutrient solutions were created with eleven compounds (10% iron chelate DTPA, calcium nitrate, copper sulfate, magnesium sulphate, manganese sulphate, monopotassium phosphate, potassium nitrate, sodium molybdate, sodium borate, zinc sulfate, and nitric acid), which are commonly used as mineral fertilizers in commercial hydroponic production systems. These compounds are known to provide essential nutrients for plants in hydroponic systems (Salisbury & Ross, 1994). To prepare the synthetic hydroponic nutrient solutions, random combinations of the eleven compounds were dissolved in water. The concentration of each compound was determined based on their nutrient compositions and optimal nutrient concentrations reported by multiple authors (Aini et al., 2019; Domingues et al., 2012; Trejo-Téllez & Gomez-Merino, 2012). The concentration of each compound in each synthetic sample used in this study was determined by independently sampling from uniform distributions ranging from zero to twice the optimal concentration of each compound. The concentration distributions for each macronutrient present in the synthetic hydroponic nutrient solutions is shown in Figure 1 below.

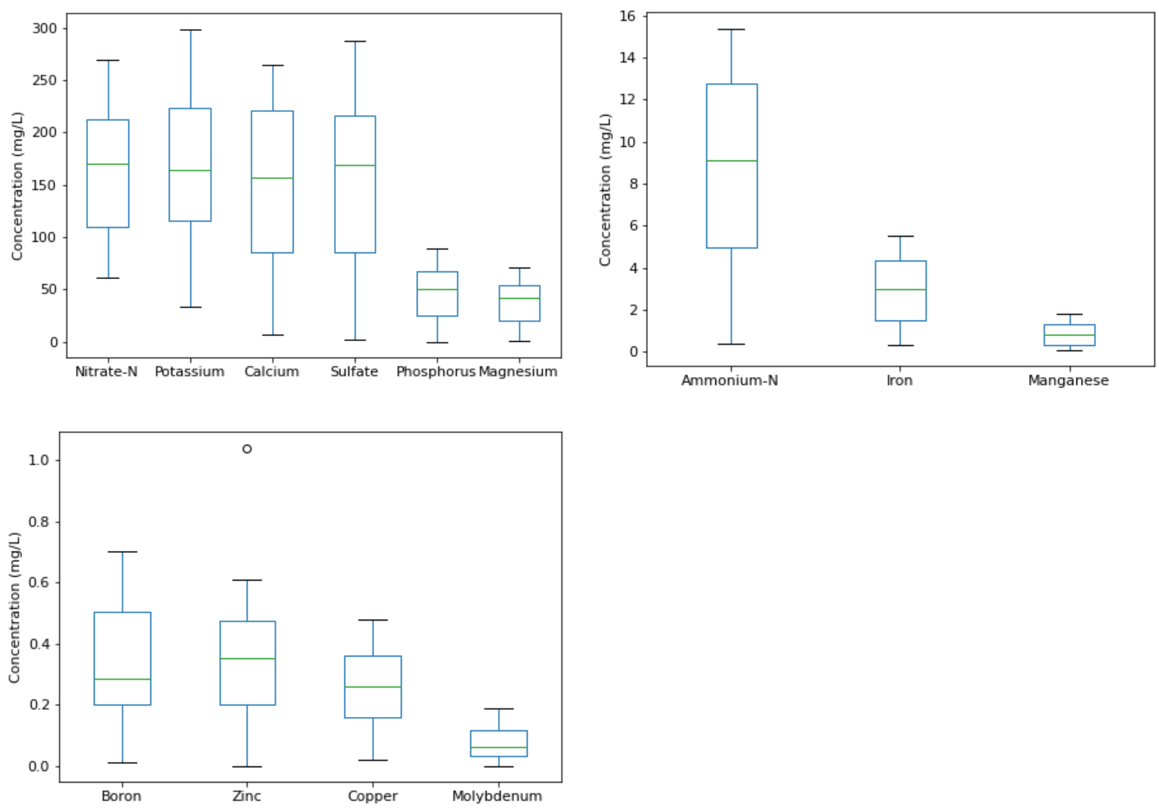

Figure 1: Concentration distributions of nutrients present in the forty-six synthetic hydroponic nutrient solutions used for this study.

The synthetic hydroponic nutrient solutions were created by dissolving these compounds in 50 mL of tap water. Tap water was used to better represent the characteristics of real hydroponic samples. Any compound added with a mass over 14 mg was weighed using an analytical balance and added into the solution directly. When the mass to be added was under 14 mg, the corresponding volume of stock solution was pipetted and mixed into the solution. Stock solutions were created by weighing out approximately 100 mg of the compound and dissolving in 50 mL of water, leaving a concentration of approximately 2000 mg L^{-1}. The concentrations were then used to determine the volumes of stock solution that needed to be added to supply the required mass of the compound. The minimum volume of the pipettes was 10 μL. When the volume needed to be added was below the minimum limit, a dilution of the stock solution was made so that the required mass of compound could be added to the solution using a volume greater than 10 μL.

Spectrophotometric Methods

The forty-six hydroponic nutrient solutions were individually analyzed with the GatorSpec and the NanoDrop2000C. To obtain values for absorbance, the intensity of light passing through the sample and a reference is required. The reference used for each analysis was deionized water. The machines measured the intensity of UV-Vis light passing through a 1 cm path of the reference or sample within a quartz cuvette. Absorbance was calculated from measured intensity with Equation 1, where I_O is the intensity of light passing through the reference, and I is the intensity of light passing through the sample (Mayerhöfer, 2020).

$$A = \log_{10} \frac{I_O}{I} \tag{1}$$

The GatorSpec is a low-cost, custom-built, open-source spectroscopy setup that was built by Joe Barrett Carter, a graduate student University of Florida Department of Agricultural and Biological Engineering. The setup is shown in Figure 2 and components are listed in Table 1.

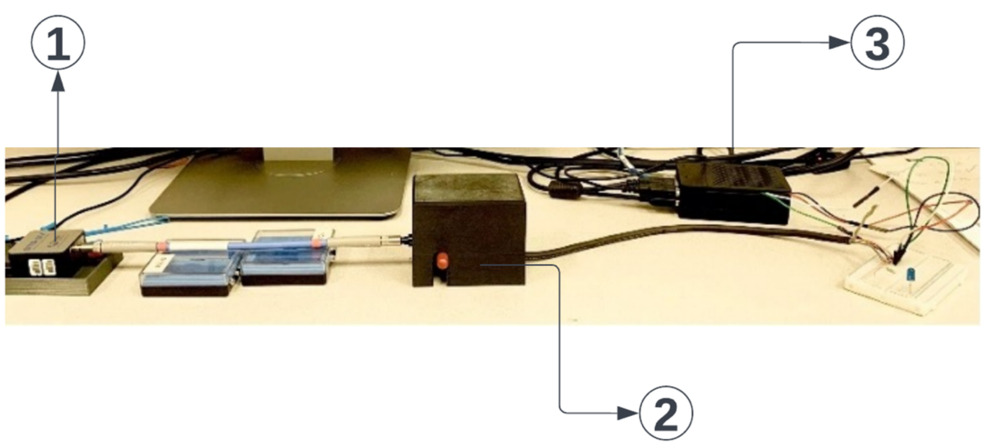

Figure 2: The GatorSpec benchtop spectroscopy setup. See Table 1 for numbering key.

Table 1: Main components of the GatorSpec benchtop spectroscopy setup

Label	Part Name	Manufacturer	Model Number
1	Minatare UV-Vis Spectrophotometer	Ocean Insight	STS-UV-L-10-400-SMA
2	Xenon Flash Lamp in 3-D printed enclosure	Hamamatsu	L13651-01
3	Raspberry Pi Microcomputer	Raspberry Pi Foundation	Raspberry Pi Model 3B

The samples were analyzed on the GatorSpec using a programmed control system and interface, and an integration time of 23 milliseconds was used for each measurement. The GatorSpec has a spectral range that goes from 184.2 nm to 663.1 nm, and a resolution of 0.45 nm. Meanwhile, the NanoDrop has a spectral range of 190-840 nm and a resolution of < 1.8 nm.

Absorbance data was collected for diluted and undiluted samples. A 1:30 dilution was performed for each sample, as that was the midpoint between zero absorbance and the maximum absorbance that could be recorded by the system for a sample with ideal nitrate concentration. Nitrate is present in high concentrations within hydroponic nutrient solutions, so the samples were diluted because it is a strong absorber of light and causes absorbance to saturate and plateau as concentration increases (Mayerhöfer, 2020).

The theoretical concentration of nitrate in the samples was determined using stoichiometry based on the amount of each compound containing nitrate-nitrogen that was added in each solution. These compounds were calcium nitrate, potassium nitrate, and nitric acid.

Data Analysis Methods

Principal component analysis (PCA) was performed using the sci-kit learn library in Python on the absorbance datasets from both instruments, the GatorSpec and NanoDrop 2000C. PCA is an unsupervised statistical technique that analyzes a dataset with many dependent variables and captures the most important information from the data to express it in fewer variables called principal components (Abdi & Williams, 2010). The goal of PCA is to reduce the dimensionality of a dataset by transforming a larger set of variables into a few principal components while preserving as much information as possible (Ringner, 2008). PCA performed on both the diluted and undiluted samples was used to determine the most meaningful dependent variables/features, and in this case, wavelengths, to re-express the data (Shlens, 2014). PCA results in a loading vector corresponding to each principal component and which has the same length as the original dataset. Loading vectors for the first principal components were used to compare the two spectroscopy machines being compared in this study. PCA is an unsupervised analysis, indicating that no information about the target nitrate concentrations is used.

A Partial Least Squares (PLS) Regression was also performed using the sci-kit learn library in Python to determine the spectrophotometers' abilities to accurately predict the true nitrate concentrations in the samples based on spectral absorbance. Multiple compounds affect absorbance, and it is difficult to parse out the effects of each compound within chemically complex solutions on the absorbance. Each compound also affects absorbance at multiple wavelengths, and it is difficult to determine which wavelengths are necessary to estimate chemical concentrations. PLS predicts the dependent variables, and in this case nitrate concentrations, from a large set of independent variables, which are the wavelengths (Abdi, 2003). PLS is a supervised version of PCA, indicating that it takes into account the target nitrate concentrations when determining the weights of each principal component. For this analysis, 70% of the absorbance data was used as training data for the model, while 30% of the data was used to test the model and predict the nitrate concentrations in the samples. PLS was performed with the training data and the model was fit to the data. 5-fold cross validation was used on the training set to determine the best number of components for the PLS model. Predicted values for nitrate concentrations in each sample were obtained with the absorbance data of the test set. These values were compared with the theoretical nitrate concentrations of the test set using linear regression and by calculating performance metrics (r^2 and RMSE).

Results and Discussion

Principal Component Analysis

The first principal component in a PCA explains most of the variability in the data (Ringner, 2008). Figure 3 shows a direct comparison between the first principal components derived from the UV-Vis absorbance of diluted samples and undiluted samples. It also shows the "target" nitrate concentrations in the samples represented with color gradient dots. There was a much stronger correlation in the undiluted sample data, indicating that the variability in the NanoDrop2000C undiluted data and GatorSpec data is very similar. Alternatively, the principal components of the absorbance of diluted samples are loosely correlated. However, both of the relationships shown in Figure 3 are statistically significant, as the p-values for the regression are well under 0.05. Also, there is a correlation between the target nitrate concentrations and the first principal components derived from the diluted samples as indicated by the shading in Figure 3 and confirmed in Figure 4, and this relationship is not present for the undiluted samples.

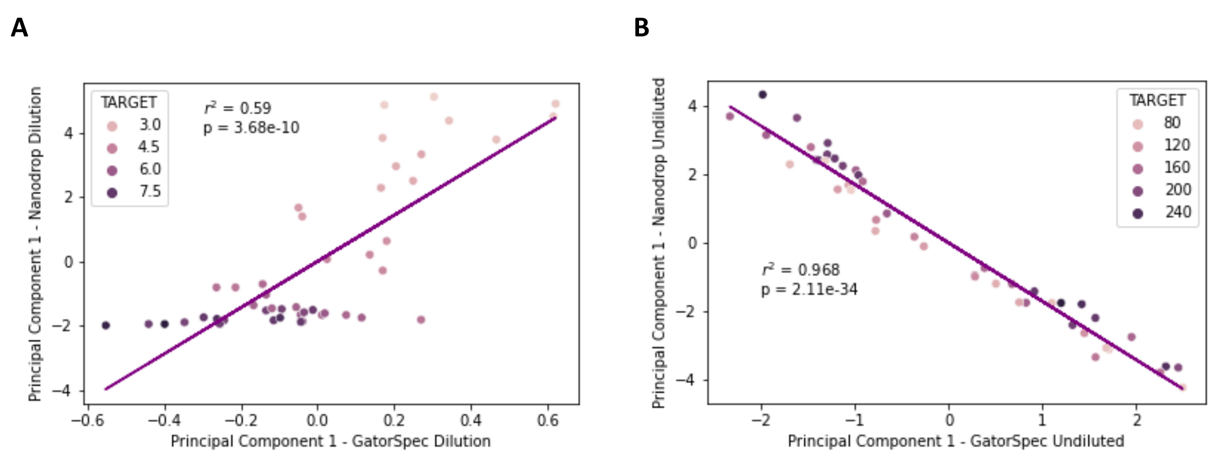

Figure 3: Principal Component 1 Comparison for (A) Diluted Samples and (B) Undiluted Samples

The relationship between the first principal component and the target nitrate concentration was examined more closely. As shown in Figure 4, there is a strong correlation between the first principal component and the target nitrate concentrations for the diluted samples analyzed by both machines. There is also no significant relationship ($p = 0.26$ and $p = 0.69$ for data produced by the NanoDrop and GatorSpec, respectively) between the first principal components of the data produced by the analysis of undiluted samples by each machine and target nitrate concentrations.

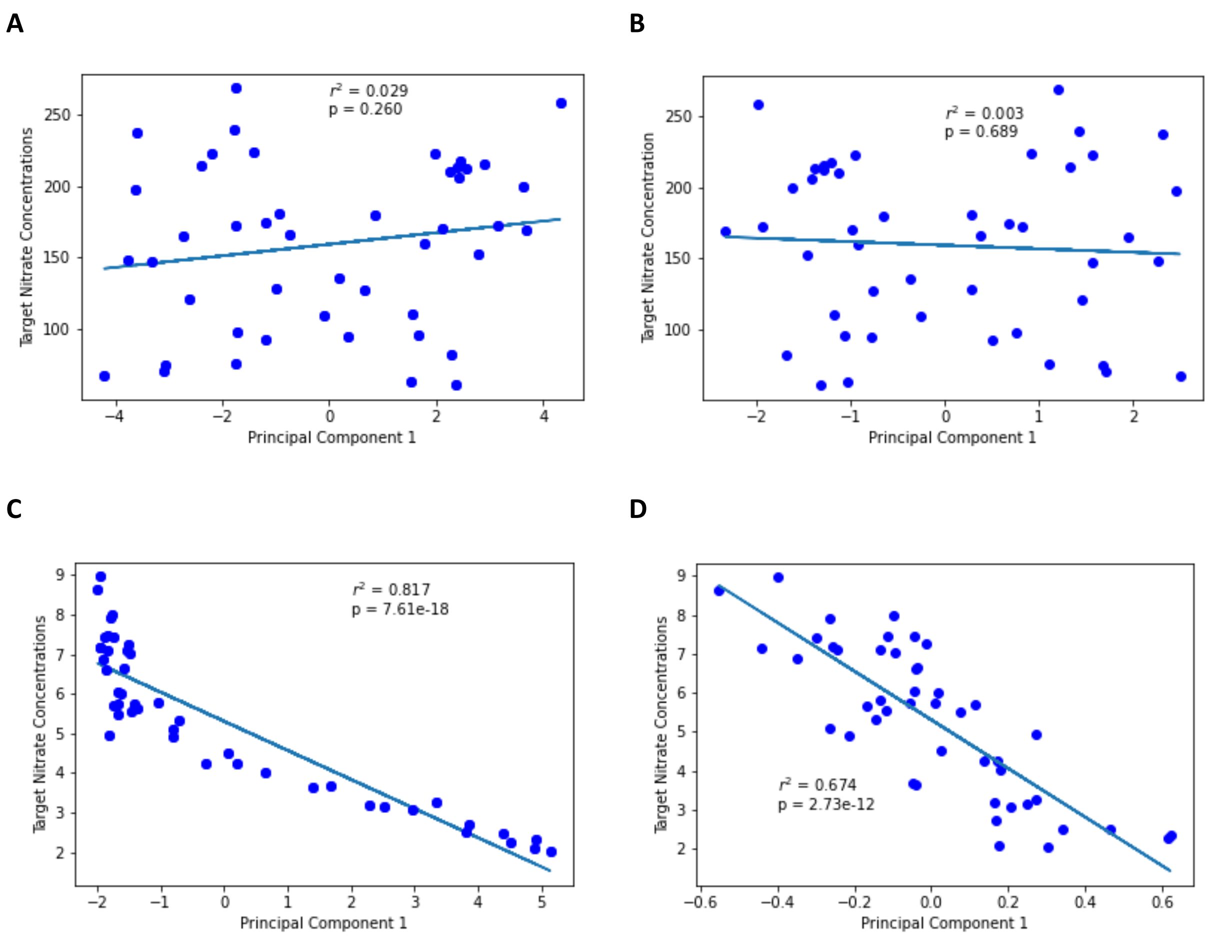

Figure 4: Target nitrate concentrations versus the first principal components produced by the UV-Vis analysis of (A) undiluted samples by the NanoDrop, (B) undiluted samples by the GatorSpec, (C) diluted samples by the NanoDrop, and (D) diluted samples by the GatorSpec.

Having the same trend occur with an unsupervised analysis for both the NanoDrop2000C and the GatorSpec indicates that based off the absorbance data alone, the information content of the data was very comparable. Both spectroscopy setups were compared in relation to the dependence of the absorbance data on the properties of the solution, and the outcome was very similar. The data independently has the same relationship with the nitrate concentrations in the samples, helping validate the GatorSpec's absorbance data. Interestingly, for the analysis of diluted samples, there is a stronger correlation between the first principal component of the NanoDrop data and nitrate concentration compared to that of the GatorSpec. Also, while both are correlated to nitrate concentration, the first principal components derived from the UV-Vis absorption of diluted samples are less correlated than that of the undiluted samples. This suggests that the two machines have diverging sensitivities at lower nitrate concentrations.

It was also determined by PCA which wavelengths contribute the most to the variance of the absorbance data (see Table 1). Nitrate absorbs light around 210-220 nm, but absorbance in this range can also be affected by other solutes present in complex natural solutions (Armstrong, 1963). For the diluted samples, the wavelengths that contribute the most to each principal component is near this range. For diluted samples, variability of the data produced by the NanoDrop was most affected by a wavelength the range of peak absorption by nitrate, while principal wavelengths for the GatorSpec were slightly above this range. The principal wavelengths for the undiluted samples were significantly above the range of peak absorption by nitrate, indicating that the effects of nitrate are saturated at higher concentrations, and the variability in the absorbance data for undiluted samples was likely due to the effects of other compounds in the samples. Both machines gave similar PCA results.

Table 2: Principal Component Analysis

Sample Type	Principal Component	Wavelength	Explained Variance (%)
GatorSpec Diluted	PC1	229.4	65.47
	PC2	228.9	24.58
NanoDrop Diluted	PC1	219	97.37
	PC2	194	1.11
GatorSpec Undiluted	PC1	261.3	83.70
	PC2	242.6	7.81
NanoDrop Undiluted	PC1	244	81.45
	PC2	240	6.79

The explained variance in a principal component analysis is the percent of variance in the dataset that is attributed by the corresponding principal component. Table 1 shows that the first principal component for the undiluted samples encapsulates the same percentage of variance for both spectroscopy setups. For the diluted samples, most of the explained variance is in the first principal component for the NanoDrop, while it is more split between the first and second principal component for the GatorSpec. Since there is a strong correlation between the first principal component and the target nitrate concentrations for the diluted samples, the majority of variance is explained by the first principal component, and the variation in these components is caused by wavelengths close to the range of peak nitrate absorption, it can be determined that the variance in the absorbance data is attributed to the nitrate concentrations in the diluted samples. Similarly the results for the analysis of undiluted samples indicate that the spectral absorbance of undiluted samples is less correlated with the nitrate concentrations. Most importantly to the objective of this study, the PCA results produced by the two machines are in very close agreement.

Partial Least Squares Regression

Since the principal component analysis is unsupervised and is not dependent on the target nitrate concentrations, a partial least squares regression was performed to see how accurately the nitrate concentrations in the samples is predicted by the absorbance data produced by each machine. For the diluted samples, the model was highly accurate in predicting the true nitrate concentrations. As shown in Figure 5, the root mean squared error was 0.633 and 0.216 mg L^{-1} for the NanoDrop and GatorSpec, respectively, indicating good performance of prediction by both machines with that of the GatorSpec being slightly better than that of the NanoDrop

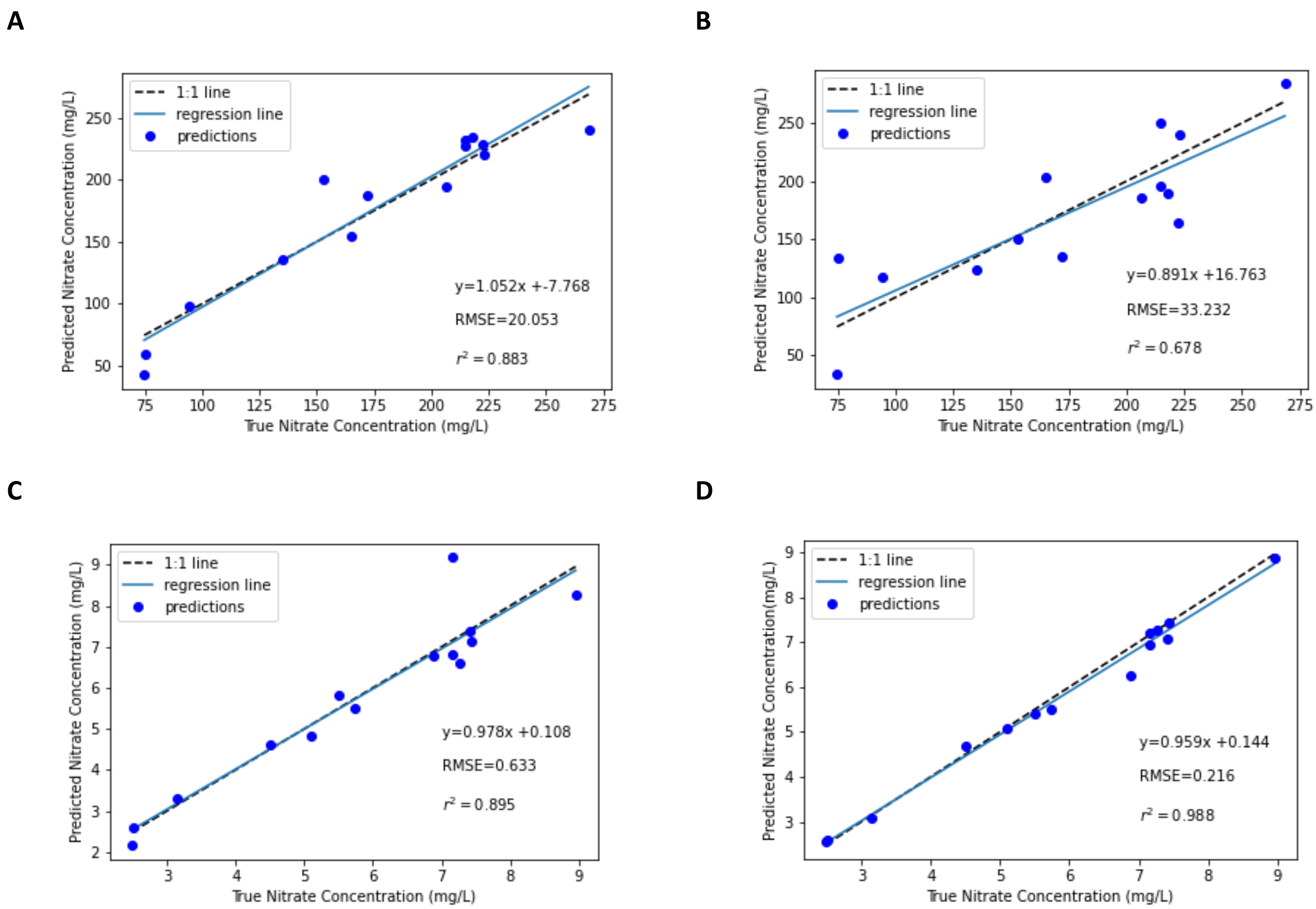

Figure 5: PLS results for predicting nitrate concentrations based on the absorbance of (A) undiluted samples produced by the NanoDrop, (B) undiluted samples produced by the GatorSpec, (C) diluted samples produced by the NanoDrop, and (D) diluted samples produced by the GatorSpec.

The r^2 values corresponding to the undiluted samples are lower, indicating that the models do not predict the theoretical nitrate concentrations as accurately as those derived from diluted samples. Additionally, the model derived from the analysis of diluted samples by the GatorSpec had the best performance and showed the most improvement caused by sample dilution. From the PCA results, the variance was the most spread out between the first two principal components for the absorbance of diluted samples produced by the GatorSpec. This dataset could have resulted in the best predictive model because of this division of variance between multiple components to be considered independently in the regression model. The NanoDrop had better results for the undiluted samples while the GatorSpec had better results for the diluted samples. Overall, the models based off the absorbance values measured by both machines were able to accurately predict the theoretical nitrate concentrations.

Conclusions

In conclusion, the GatorSpec collected reliable absorbance data from forty-six synthetic hydroponic nutrient solutions, and this low-cost setup can be used to accurately predict nitrate concentrations in hydroponic nutrient solutions. PCA results indicated that the absorbance data from both spectroscopy setups provide similar information, and PLS results showed that a model created from the absorbance data can accurately predict the theoretical nitrate concentrations in the diluted samples for both machines. Further research could be done to perform a similar analysis predicting other main nutrients in hydroponic solutions, such as potassium, calcium, and sulfate to expand this application to measuring other environmental water quality parameters with UV-Vis spectroscopy. Using the GatorSpec to form the technological basis for developing field-deployable water sensors is being explored as a way the system could be expanded in the future. This analysis could be improved in multiple ways. One way would be with a larger sample size to create better models, and another would be with a larger variety of chemical constituents and concentration values to expand this analysis to sample types beyond hydroponics such as irrigation systems and stormwater runoff. This analysis could also be tested on real hydroponic nutrient solutions found in commercial hydroponic systems, not just synthetic samples made in a laboratory setting. Overall, the GatorSpec has a comparable performance to that of the NanoDrop2000C and functions well as a UV-Vis spectrophotometer. The validation of the GatorSpec opens the door for many applications of this low-cost water quality analysis system such as improved hydrological modeling and water quality monitoring. In addition to hydroponic plant producers, having open-source water quality analysis can benefit people ranging from conventional farmers to homeowners to stormwater managers by allowing them to collect and share data more easily. This increase in data availability allows water system managers to have access to more information to make more efficient and timely decisions.

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