Seneca APPLIED RESEARCH

February 23rd, 2021

PI: George Clark

RA: Jasmin Khan

Company: Just Vertical

Table of Contents

Abstract 3

Introduction 3

Methods 4

Initial Screening to Determine the Best Dilution Factor for ISE 4

ISE Analysis to Determine NO3/N content in Basil and Mint 5

Initial Screening to Determine the Best MWD Parameters 5

Initial Screening to Determine the Best Dilution Factor for ICP 6

Screening Results 6

Summary of the Best Dilution Factor for ISE 6

Summary of the Best Parameters for Microwave Digestion 7

Summary of the Best Dilution Factor for ICP-OES 7

ICP-OES Analysis to Determine Mineral content in Basil and Mint 7

ICP-OES Results Obtained for the Horizontal Units 9

Results Obtained for the Vertical Units 11

Conclusions and Implications 18

Future Research Considerations 19

References 20

8.0 SOPs 21

8.1 Standard Operating Procedure for Total Ionic Strength Adjustment Buffer (TISAB) Solution 21

8.2 Standard Operating Procedure for Determination of Dry Plant Content 23

8.3 Standard Operating Procedure for Determination of NO_3/N content using Ion Specific Electrode (ISE) 25

8.4 Standard Operating Procedure for Extraction via Microwave Digestion 28

8.5 Standard Operating Procedure for Operating the ICP-OES 30

Appendix 33

Kale Samples 33

Pictures Throughout Project 36

Graphs for the various watering cycles and its lighting regime 40

Figures and Tables to compare pH and EC for each watering cycle and lighting regime within the various units 47

Abstract

Plants are a natural source of vitamins, minerals, and many other essential nutritional factors. From basil, mint to kale and other leafy greens and flower plants the Just Vertical AEVA units are able to grow them to their full potential with all their nutritional benefits. The association between Seneca College Innovation and Just Vertical will allow for the determination of the best parameters to grow basil and mint to their full potential, by exceeding concentrations for their macronutrients and micronutrients. The scope provided for the inorganics section of the project looks at the most common minerals by analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP- OES) and analysis of the total extractable nitrogen content of the plants via a Nitrate Ion Specific Electrode. Grocery store samples were also analyzed in contrast to the plants harvested on campus through Just Vertical AEVA vertical farming units along with horizontal growth farming. Additionally, this study also completed an analysis of Basil and Mint samples harvested in Timmins, to determine if there is an observable difference of the concentration of minerals and total nitrogen content with respect to travel time. These inorganic studies, combined with observation and detailing of the various lighting and watering growth parameters including added nutrients, pH, EC< humidity, temperature, plant height and root mass and travel time within the vertical and horizontal two-week growth cycles of basil and min, can offer insight into the overall efficacy of the AEVA units and horizontal units to grow basil and mint.

Introduction

Just Vertical is an indoor, vertical farming company, that builds, sells and supports indoor growing gardens and farming modules. Seneca College's collaboration with Just Vertical helps to ensure that the overall health of the plants can provide the most beneficial nutrimental factors for Just Vertical's clients. The inorganics portion of the project looks at the most common micronutrients and macronutrients of the plants along with the overall nitrogen content. The micronutrients and macronutrients are established through the most common minerals found within fruits and vegetables. Some of these common nutrients include Calcium (Ca), Iron (Fe), Magnesium (Mg), Potassium (K), Sodium (Na), Sulphur (S), Zinc (Zn) and more. The overall nitrogen content of the plant, allows one to conclude the general health of the plant beyond a physical examination. Since the plants are grown within the indoor vertical hydroponic farming system, there is a limited number of parameters that can be adjusted allowing for enhancement of the nutrient contents. Lighting and watering cycles of the plants are the two biggest parameters that is altered throughout the entire duration of the project. Every two weeks, the parameters are altered allowing for a different growth cycle to impact the nutrimental factors. Added nutrients are required for the growth of the plants which are

constantly monitored to a have a pH of 5.8 – 6.2 and an EC of 1.2 – 1.5 mS/cm. The temperature of the environment and the humidity are also recorded to determine if a fluctuation may impact the overall plant.

Ocimum basilicum (Basil) and *Mentha x piperata* (Mint) are the two plants that are grown, examined and analyzed throughout the project. These plants were chosen due to their short growth time as well as their fast turnover rate. To determine the overall nitrogen content of the plants, a membrane specific probe was bought so that nitrate can be extracted and further calculated to determine the total extractable nitrogen content. To determine various minerals within the plant samples, microwave digestion (MWD) followed by inductively coupling plasma – optical emission spectroscopy (ICP - OES) was used to prepare the samples and analyze each analyte individually in triplicates.

Methods

Initial Screening to Determine the Best Dilution Factor for ISE

The method to determine the NO_3/N content in plants was undertaken using a method detailed in the *Handbook of Reference Methods for Plant Analysis* (Karla, 1998). The methodology utilizes a nitrate (NO_3) ion specific electrode (ISE) that contains a membrane to allow for detection of nitrate, which can be used as a measure for the determination of the extractable total nitrogen content within the plant. The solution used to extract nitrate from the plant sample is also an ionic strength adjustment buffer required for ISE studies and is made up of different chemical reagents. Specifically, the extraction buffer is comprised of aluminum sulphate, boric acid, sulphamic acid and silver sulphate, all within the ratio of 6.7 g, 1.2 g, 2 g and 1 g, respectfully. A combination of these reagents dissolved in water becomes the medium for the extractable solution of nitrate from the plant material. For this analysis, a mass of 0.5 g of plant matter including stem and leaves was weighed, ground to a fine particle size and mixed with 25 mL of Extraction/TISAB solution for 30 minutes on a mechanical shaker. Once the mixing has completed, the sample is filtered using a vacuum filtration with a Whatman 4.0 filter, (see Figure 1.0 -2.0 in the Appendix). To determine the most efficient dilution factor, tests were made using 1:5, 1:10 and 1:100 dilution with the extraction buffer and then analyzed with the ISE.

Prior to analysis upon the ISE, a calibration curve was created from a stock solution of 1000 ppm nitrate using potassium. The calibration curve used to determine the best dilution factor for the plants were 5 ppm, 20 ppm, 50 ppm and 100 ppm NO_3/N, where each standard was diluted with the extraction/buffer solution. An instrumental Nerst-

slope within the 90.0% - 100.0% of the theoretical ideal was achieved with these standards prior to analysis of the dried sample with the different dilution factors.

ISE Analysis to Determine NO3/N content in Basil and Mint

The samples were stored in the freezer at -80°C after harvest. Prior to analysis, the samples were taken out of the freezer and allowed to air dry to room temperature. Once the samples had equilibrated to room temperature (after approximately 30 minutes), the samples were weighed, placed in aluminum cups and dried in an oven at 70°C for 2 hours. The samples were then placed directly into a desiccator for 1 hour. Once the drying of the samples was completed, they were ground to a fine particle size. The samples were mixed with 25 mL of extraction/TISAB buffer for 30 minutes on a mechanical shaker. Once the mixing completed, the samples are filtered using a vacuum filtration with a Whatman 4.0 filter paper. All samples are diluted with extraction/TISAB buffer solution to the best dilution factor of 1:10. A calibration curve of 5 ppm, 20 ppm and 50 ppm NO_3/N were used and made prior to every analysis.

Initial Screening to Determine the Best MWD Parameters

ICP-OES is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to the ground state. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its chemical character. An analyst can select multiple wavelengths (and therefore elements) for analysis and after running known standards for each element, can create a calibration curve for each element of interest that can be used to qualify elemental concentration in an unknown.

ICP-OES analysis required a sample to be in solution and microwave digestion was the method selected for converting (or digesting) the solid plant material into a suitable liquid form.

Given this, *aqua regia* microwave digestion method was screened and developed after a comparative literature review of plant digestion methods, including a review of modern methods for the digestion cannabis sample. Several screening runs were undertaken, each of which included the adjusting the sample mass, reagent mixture and reagent volume to obtain optimal digestion of the plant samples. The final digestion method selected contained 3 temperature settings, ramp speeds and hold time.

The digestion method contained 3 steps with different temperatures, ramp and hold time seen in Table 1.0. The different sample masses that were analyzed were 0.25 g, 0.5 g and 1.0 g. The different types of tested reagent added to the samples were pure

concentrated nitric acid (10 mL), a mixture of concentrated nitric acid and concentrated hydrochloric (7 mL: 3mL respectively) and lastly a mixture of concentrated nitric acid, concentrated hydrochloric acid and 3% hydrogen peroxide (7 mL: 2 mL: 1 mL respectively).

Table 1.0: Microwave digestion method settings

Step	Target Temperature (°C)	Ramp Time (minutes)	Hold Time (minutes)	Power (W)
1	160	5	5	90
2	190	5	20	100
3	50	1	30	0

The best variables obtained from the microwave digestion were selected to then be used to determine the best dilution factor for analysis upon the ICP-OES (refer to SOP 8.4 and 8.5).

Initial Screening to Determine the Best Dilution Factor for ICP

Yttrium was used as the internal standard and made to an intermediate solution of 100 ppm with the 2% v/v nitric acid (trace metal grade) used as the diluent. To prepare the calibration curve, 5 ppm and 50 ppm of a customized and validated calibration standard (SCP Science) was used. This standard included the following elements Aluminum (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Sulphur (S) and Zinc (Zn). Each standard was prepared with a 1 mL addition of the 100-ppm Internal Standard solution A sample blank is prepared with the addition of 1 ppm internal standard with the 2% nitric acid diluent. As an initial screening, dried basil and mint samples were prepared to several dilutions which were diluted with the 2% nitric acid diluent at dilution factors of 1:2, 1:10 and 1:100 to determine the best dilution factor to continue carrying out the project in order to balance Limit of Detection with the sample volume/dilution to ensure repeatable analysis and adequate volume for analysis.

Screening Results

Summary of the Best Dilution Factor for ISE

Three different types of dilutions were analyzed to determine the best dilution factor for ISE, 1:2, 1:10 and 1:100. Of note, the raw samples had a distinct faded yellow colour. When the dilution factor of 1:2 was analyzed, the concentration obtained through the probe was significantly higher than the highest standard of the prepared calibration

curve. When the dilution factor 1:100 was analyzed, the concentration obtained through the probe was above the limit of detection of the instrument (the lowest analyte concentration likely to be distinguished from the blank) but within the linear range of concentration of the prepared calibration curve. To ensure that the concentration obtained from the sample reading was withing the linear portion of the calibration curve, allowing for error, a 1:10 dilution factor was determined as the best dilution factor for the NO_3/N content ion specific electrode analysis.

Summary of the Best Parameters for Microwave Digestion

There were several parameters considered when digesting the plant samples prior to ICP-OES analysis. A common generic microwave digestion method for plant material that was initially referenced utilized 500 mg of plant sample that was digested in 10 mL of concentrated nitric acid. Plant samples that contained 250 mg, 500 mg and 1 g of sample in 10 mL of concentrated nitric acid did not allow for sufficient digestion of the sample. Throughout the analysis of all the samples with the different parameters, it was concluded that 250 mg of sample along with 7 mL of concentrated nitric acid, 2 mL of concentrated hydrochloric acid and 1 mL of 3% hydrogen peroxide, resulted in the most optimal digestion of the dried plant samples.

Summary of the Best Dilution Factor for ICP-OES

After the best parameters for microwave digestion were established, several dilutions factors were analyzed, 1:2, 1:10 and 1:100. When the dilution factor of 1:100 was looked at, all the elements had a concentration that was near limit of detection (LOD) and Phosphorus along with Sulphur were below LOD. When the dilution factor was 1:2, Calcium and Potassium were detected above the calibration curve, despite the other elements being within the curve. When the dilution factor was 1:10, all elements were within the calibration curve except for Sulphur. Based on this evaluation of these three different dilution factors, it was concluded to use a dilution factor of 1:5, as this dilution factor resulted in the best balance of the majority of the analyzed elements being above the Limit of Quantification (LOQ) of the instrument.

ICP-OES Analysis to Determine Mineral content in Basil and Mint

Prior to ICP-OES analysis, microwave digestion was completed on plant samples using its stems and leaves. The reagents utilized for the microwave digestion includes *aqua regia* and 3% hydrogen peroxide for a total volume of 10 mL. The ICP-OES was optimized to its best parameters (refer to SOP 8.5). Each element was analyzed at the wavelength with the least number of interferences, with Aluminum at 396.152 nm, Boron at 249.772 nm, Calcium at 396.847 nm, Copper at 327.395 nm, Iron at 238.204 nm, Potassium at 766.491 nm, Magnesium at 279.553 nm, Manganese at 257.610 nm,

Sodium at 589.592 nm, Phosphorus at 213.618 nm, Sulphur at 181.972 nm and Zinc at 213.857 nm. The standard calibrations used were 2 ppm, 20 ppm and 50 ppm. All samples were spiked with 1 ppm of the internal standard Yttrium and diluted with 2% nitric acid.

Of particular note in this study was the unfortunate lack of precision and accuracy for the quantification of sulphur. Although ICP-OES methods for the analysis of sulphur have been used since the 1990s, sulphur emission signals are in the ultraviolet region (181.972 nm) and are therefore very susceptible to interferences. Traditionally, digestion methods that are optimized for the recovery of sulphur have included perchloric and/or hydrofluoric acid, which have inherent safety concerns.

During the screening and method development of the ICP-OES study, it was observed that although sulphur concentration could be determined with the sample preparation method and instrumental parameters chosen, the resulting concentration data generated by the ICP-OES for sulphur typically fell between the Limit of Detection (LOD or the lowest quantity or concentration of sulphur that can be reliably detected but not necessarily quantified with a given analytical method) and the Limit of Quantification (LOQ or the lowest quantity or concentration of sulphur, that can be reliably quantified with a given analytical method), likely the results of its low wavelength of analysis and susceptibility to interferences. The reason for this is twofold:

1/ To render emission counts for sulphur in the plant sample to be higher and therefore above the LOQ for sulphur, more mass of plant sample would be required for each analysis. However, given the limited biomass that could be generated by the units within the growing cycle and that only a portion of this was available for ICP-OES studies, this was not feasible given the multi-faceted requirements of the overall study.

2/ The multi-element nature of this analysis required a balance to be attained between biomass available for analysis, sample preparation time and method, instrumental scheduling, and instrumental method development requiring that all elements of interest render data that is above the LOQ for each element and can therefore be reliably and reproducibly quantified. With this in mind, to optimize this analysis for sulphur recover (i.e., more aggressive digestion methods using a different suit of chemical that might have a safety concern and/or a different suit of instrumental parameters) would likely render the recovery of the balance of the remaining elements to be non-ideal. Given this, a decision was made to optimize the recovery of the majority of the elements of interest with the understanding that the data gleaned from sulphur would be sub-optimal.

However, it should be noted that although the reliable and absolute concentration of sulphur could not be determined with confidence given that all of the samples analyzed were below the LOQ of sulphur using the final methods selected, the *overall relative trending* of sulphur concentration could be determined as most of the samples analyzed were determined to be above the LOD for sulphur using the method selected. This data has been summarized below.

ICP-OES Results Obtained for the Horizontal Units

The mineral and elemental concentration results obtained from the Horizontal units can be seen in Tables 2.0 and 3.0, for basil and mint, respectively. These data tables are formatted in such a way that within each harvested date, the column on the left are plants harvested from the top shelf of the unit (**HTB/M** – Horizontal Unit Top Shelf – Basil/Mint) and the column on the right are plants harvested from the middle shelf of the unit (**HMB/M** – Horizontal Unit Middle Shelf – Basil/Mint). Ultimately, the two shelves allow for more yield and reproducibility for its specific parameter.

Concentration for Aluminum (% w/w) and Copper (%w/w) are below LOD (note that this might not be due to their absence within in the sample but due to the fact that this method utilized a lower mass of plant matter for each sample analyzes due to lower plant yield produced from the indoor farming units, rendering these elements undetectable).

Other elements such as boron, iron, manganese and zinc are present in very low concentrations as an abundant amount of these minerals are not generally found within the plants.

It is also indicated that although these macro/micronutrients are essential for a person, a large intake is not required for the body's function. Sulphur is highlighted in blue due to signifying that there is sulphur present within the plants but due to utilizing a lower mass for microwave digestion, but detected as just below the limit of quantification (LOQ). Therefore, as the values are not precise, they should be looked as a trend rather than quantitative values.

The concentration of nitrogen is included in the tables to allow a contrast with each mineral depending on the harvest along with the growth parameters. The variation in the lighting and water parameters are 2 main variables that impacts the overall growth of the plants and concentrations of minerals that each plant contain. To obtain many different growth parameters within the short period of time, and possibly have reproducible data, two different shelves within the horizontal unit were used. Grocery bought samples were obtained from Metro, the Metro brand as well as their Irresistible brand, to compare the minerals obtained with freshly harvested against grocery samples. In Table 6.0, basil and mint sample were harvested from a local farm in Timmins and brough to the Seneca at York campus, to analyze for the sample minerals to have another comparison, between freshly harvested against local farm. The graphs below Table 6.0, indicates comparison between the horizontal, vertical, Timmins and Metro samples for the different lighting and watering parameters.

Table 2.0: Qualitative Data for Basils grown on the Horizontal Units with Comparison against Grocery Samples in %w/w

Minerals	Metro	Irresistible	Oct 8		Nov 12		Nov 26		Dec 10		Dec 22		Jan 7		Jan 21	
Al	LOD	0.004	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
B	LOD	0.002	0.001	0.002	0.001	0.002	0.004	0.007	0.002	LOD	LOD	LOD	0.0016	0.0015	0.0018	LOD
Ca	1.5	1.85	0.92	1.52	0.59	0.82	1.12	1.18	0.48	0.43	0.44	0.78	0.81	1.07	0.67	0.51
Cu	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Fe	0.01	0.01	0.004	0.01	0.002	0.003	0.01	0.01	LOD	LOD	LOD	LOD	0.01	0.009	0.03	0.0049
K	3.40	2.93	2.74	4.09	1.27	1.98	3.48	3.77	2.27	2.59	3.03	3.43	3.22	4.27	3.44	3.12
Mg	0.64	0.50	0.45	0.75	0.25	0.34	0.51	0.60	0.37	0.33	0.37	0.35	0.34	0.47	0.31	0.20
Mn	LOD	0.005	0.005	0.008	0.002	0.004	0.001	0.001	0.003	0.002	0.004	0.005	0.00076	0.00094	0.0078	0.0062
Na	0.17	0.001	0.08	0.20	0.01	0.02	0.07	0.08	0.03	0.04	0.05	0.04	0.04	0.04	0.009	0.004
P	0.65	0.29	0.32	0.88	0.20	0.26	0.67	0.67	0.39	0.42	0.49	0.52	0.51	0.52	0.44	0.35
S	0.00135	0.00425	LOD	0.001	LOD	LOD	0.0027	LOD	LOD	LOD	0.002	0.002	0.0013	LOD	0.0013	0.0011
Zn	0.03	-	0.005	0.009	0.00	0.001	0.006	0.006	LOD	LOD	LOD	LOD	0.0027	0.0028	0.002	0.0013
N	0.09	0.13	-	-	0.94	0.92	1.30	1.17	0.25	0.24	0.32	0.35	0.30	0.17	0.9	0.43
Shelf			HTB	HMB	HTB	HMB	HTB	HMB	HTB	HMB	HTB	HMB	HTB	HMB	HTB	HMB
Light			18/6	18/6	24/0	24/0	16/2	16/2	16/2	16/2	16/2	16/2	16/2	16/2	18/6	24/0
Watering			1	3	3	6	1	3	6	3	6	3	6	3	6	1

*LOD = sample reading below the Limit of Detection for that element
S (blue) = overall concentration trending only (below Limit of Quantitation but above Limit of Detection)

Table 3.0: Qualitative Data for Mint grown on the Horizontal Units with Comparison against Grocery Samples

Minerals	Metro	Irresistible	Oct 8		Nov 12		Nov 26		Dec 10		Dec 22		Jan 7		Jan 21	
Al	LOD	0.002	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD	LOD		LOD	LOD
B	LOD	LOD	LOD	LOD	0.001	0.001	0.008	0.01	0.002	LOD	0.001	LOD	0.0040		LOD	LOD
Ca	0.65	0.82	0.64	0.71	0.43	0.55	1.68	1.57	0.74	0.48	0.51	0.73	0.41		0.22	0.34
Cu	LOD	LOD	LOD	LOD	LOD	LOD	0.00	0.00	LOD	LOD	LOD	LOD	LOD		LOD	LOD
Fe	0.02	0.003	0.005	0.009	0.003	0.006	0.02	0.02	0.001	0.004	0.003	0.008	0.0031		0.0033	0.004
K	3.14	2.40	2.95	2.82	1.64	2.08	7.46	6.73	6.47	3.70	3.91	5.56	2.50		1.87	2.34
Mg	0.41	0.34	0.42	0.49	0.31	0.33	1.30	1.00	0.96	0.33	0.66	1.23	0.26		0.19	0.26
Mn	0.002	0.004	0.004	0.003	0.002	0.004	0.01	0.01	0.01	0.005	0.004	0.009	0.0037		0.0028	0.0047

Na	LOD	0.005	0.03	0.10	0.04	0.09	0.39	0.26	0.24	0.16	0.26	0.36	0.051		0.0067	0.031
P	0.46	0.27	0.43	0.47	0.26	0.27	0.83	0.79	0.70	0.47	0.42	0.71	0.21		0.16	0.21
S	0.0013	0.004	0.001	0.0012	0.0034	0.0034	0.0039	0.0033	0.0027	0.002	0.0028	0.0038	0.0011		0.0009	0.0013
Zn	0.06	-	0.006	0.005	0.00	0.002	0.008	0.007	LOD	LOD	LOD	LOD	0.0013		0.0006	0.001
N	0.07	0.83	-	-	1.33	1.33	1.41	3.04	1.83	2.40	0.75	0.45	0.39	0.54	1.14	0.68
Shelf			HTM	HMM	HTM	HMM	HTM	HMM	HTM	HMM	HTM	HMM	HTM	HMM	HTM	HMM
Light			18/6	18/6	24/0	24/0	16/2	16/2	16/2	16/2	16/2	16/2	16/2	16/2	18/6	24/0
Watering			1	3	3	6	1	3	6	3	6	3	6	3	6	1

*LOD = sample reading below the Limit of Detection for that element
S (blue) = overall concentration trending only (below Limit of Quantitation but above Limit of Detection)

Results Obtained for the Vertical Units

The results obtained from the vertical units are seen in Table 3.0 and 4.0, for basil and mint, respectively. The tables are formatted in such a way that within each harvested date, the column on the left are plants harvested from AEVA 1 (**A1B/M** – AEVA Unit 1 – Basil/Mint) and the column on the right are plants harvested from AEVA 2 (**A2B/M** – AEVA Unit 2– Basil/Mint). Ultimately, the two units allow for more yield and reproducibility for its specific parameter. The areas within the tables for December 17 are blank due to insufficient plant yield to carry out the experiment, therefore there is no data available for that day.

Table 4.0: Qualitative Data for Basils grown on AEVA units

Minerals	Oct 8		Nov 5		Nov 19		Dec 3		Dec 17		Jan 21	
Al	LOD	LOD	0.004	0.001	LOD	LOD	LOD	LOD	LOD		LOD	LOD
B	LOD	LOD	0.002	0.002	0.00	LOD	0.004	0.002	LOD		LOD	LOD
Ca	0.90	1.08	1.57	1.40	0.70	0.42	1.22	0.58	0.51		1.19	0.83
Cu	LOD	LOD	0.00	0.00	LOD	LOD	0.00	LOD	LOD		LOD	LOD
Fe	0.02	0.01	0.001	0.001	0.003	0.004	0.009	0.005	0.001		0.0059	0.0065
K	4.26	2.83	3.90	3.37	2.05	1.80	4.95	2.27	3.05		1.89	3.00
Mg	0.31	0.39	0.47	0.39	0.20	0.12	0.58	0.22	0.38		0.33	0.30
Mn	0.01	0.01	0.02	0.01	0.005	0.003	0.009	0.002	0.004		0.008	0.0085
Na	LOD	0.03	0.02	0.01	0.008	0.02	0.07	0.02	0.04		LOD	0.01
P	0.61	0.55	0.62	0.49	0.30	0.28	0.77	0.28	0.70		0.40	0.42
S	0.001	0.001	0.0037	0.0035	0.0032	0.0034	0.0031	LOD	0.0027		0.0012	0.0010
Zn	0.01	0.02	-	-	0.001	0.00	0.007	0.003	LOD		0.0019	0.0027
N	-	-	0.83	0.45	1.28	0.81	0.34	0.40	1.07	1.74	0.22	0.67
Unit	A1B	A2B	A1B	A2B	A1B	A2B	A1B	A2B	A1B	A2B	A1B	A2B
Light	18/6	18/6	18/6	24/0	24/0	24/0	16/2	16/2	16/2	18/6	24/0	16/2

Watering	1	3	6	1	3	6	1	3	6	cts	cts	cts

*LOD = sample reading below the Limit of Detection for that element
S (blue) = overall concentration trending only (below Limit of Quantitation but above Limit of Detection)

Table 5.0: Qualitative Data for Mints grown on AEVA units

Minerals	Nov 5		Nov 19		Dec 3		Dec 17	Jan 21	
Al	LOD	LOD	LOD	LOD	0.003	0.00		LOD	LOD
B	0.001	0.001	0.00	LOD	0.007	0.01		LOD	LOD
Ca	0.59	0.48	0.48	0.36	1.01	1.35		0.28	0.41
Cu	0.00	LOD	LOD	LOD	0.00	0.001		LOD	LOD
Fe	0.004	0.001	0.005	0.004	0.009	0.001		0.0023	0.0043
K	2.87	2.79	2.21	2.85	5.04	6.61		1.33	1.65
Mg	0.31	0.25	0.26	0.14	0.69	0.86		0.14	0.26
Mn	0.008	0.005	0.006	0.003	0.008	0.01		0.0026	0.0053
Na	0.03	0.06	0.02	0.02	0.08	0.09		0.0018	0.0096
P	0.31	0.27	0.25	0.18	0.56	0.73		0.16	0.21
S	LOD	LOD	0.0041	0.0034	0.0033	0.0042		0.0008	0.0011
Zn	-	-	0.001	0.00	0.006	0.007		0.0006	0.0019
N	0.66	1.03	1.66	1.55	0.36	0.37	1.61	0.49	1.05
Unit	A1M	A2M	A1M	A2M	A1M	A2M	A1M	A1M	A2M
Light	18/6	24/0	24/0	24/0	16/2	16/2	16/2	24/0	16/2
Watering	6	1	3	6	1	3	6	cts	cts

*LOD = sample reading below the Limit of Detection for that element
S (blue) = overall concentration trending only (below Limit of Quantitation but above Limit of Detection)

Table 6.0: Qualitative Data for samples obtained from Timmins

Minerals	Basil				Mint			
Al	0.0006	0.0002	0.0007	LOD	0.0004	0.0002	LOD	LOD
B	LOD	0.0028	0.0008	0.0006	0.0010	0.0003	LOD	0.0005
Ca	0.6	1.56	0.99	0.88	0.37	0.41	0.25	0.23
Cu	LOD	LOD	0.0002	LOD	LOD	0.0002	LOD	LOD
Fe	0.0053	0.0059	0.0077	0.0040	0.0046	0.0039	0.0035	0.0073
K	1.22	3.29	3.17	2.23	0.77	1.40	0.75	1.09
Mg	0.19	0.39	0.25	0.22	0.17	0.14	0.13	0.12
Mn	0.0071	0.013	0.013	0.0011	0.0044	0.0047	0.0032	0.0028
Na	LOD	0.022	0.018	LOD	LOD	0.014	LOD	LOD
P	0.24	0.62	0.61	0.46	0.12	0.22	0.15	0.18
S	0.0007	0.0010	0.0013	0.0009	0.0005	0.0008	0.0006	0.0007
Zn	0.0006	0.0011	0.0022	0.0008	0.0003	0.0014	LOD	LOD
N	0.52	0.82	1.20	0.54	0.68	0.76	1.26	2.15
Watering	1	3	6	Continuous	1	3	6	Continuous

*LOD = sample reading below the Limit of Detection for that element
S (blue) = overall concentration trending only (below Limit of Quantitation but above Limit of Detection)

The most common lighting parameter is an 18/6 cycle as it is in-sync with a 24 hour/day cycle. The various watering cycles allow for various physical growth of the plants, but most importantly the amount of minerals that are absorbed within the plants, seen in Figure 1.0 – 4.0 for basil.

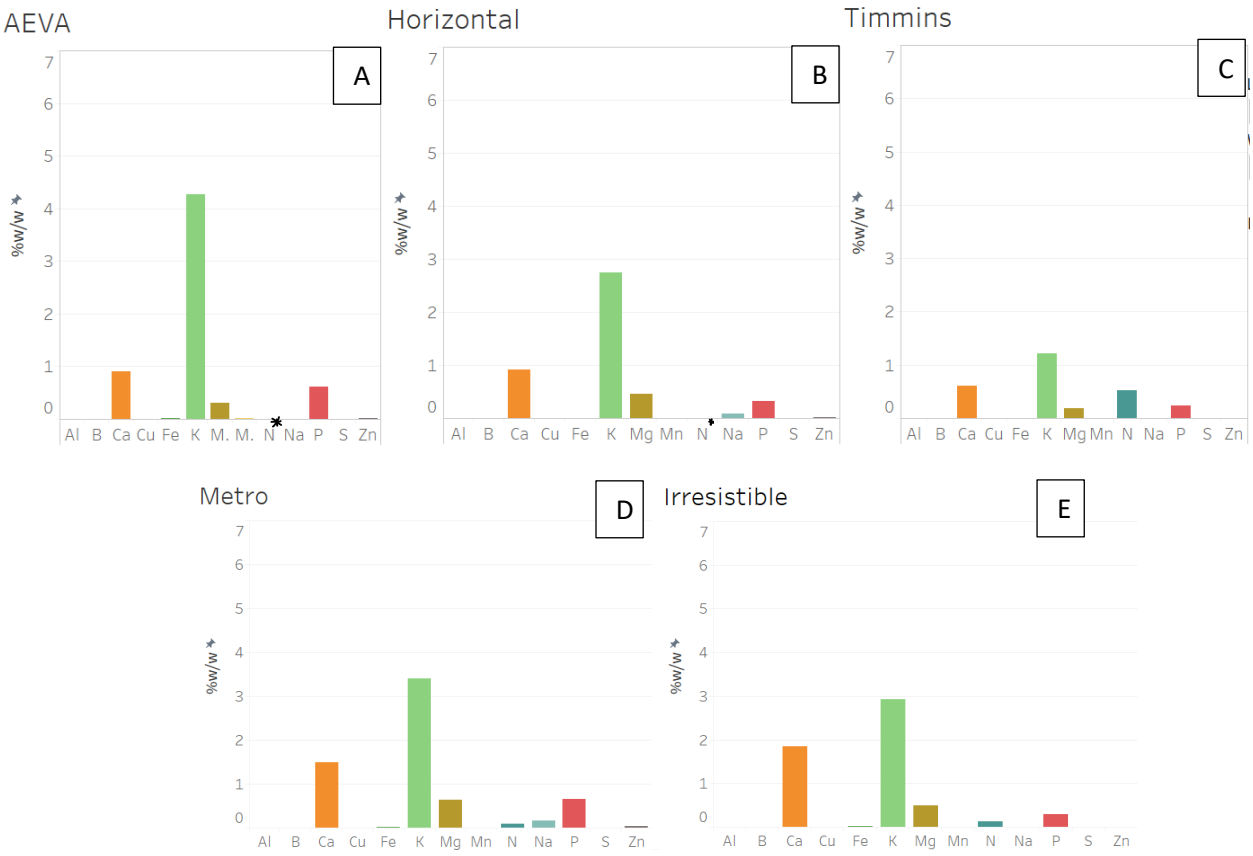


Figure 1.0 (A: AEVA, B: Horizontal, C: Timmins, D: Metro, E: Metro – Irresistible); Graphs A-C, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 1 times/day. Graph A and B*, neglects accounting for the total extractable nitrogen content due to the fact that there was limited basil yield from the harvest. Whereas, graph C are basil samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus. Graph D and E are basil samples bought from Metro, where graph D was purchased openly from the grocery stand and graph E was purchased in a package with the label “Irresistible”.

Figure 1.0 allows for the best comparison between the basil samples obtained. At an overall glance, it is evident that the AEVA unit contained a larger concentration in the primary elements such as potassium, magnesium and phosphorus. It is also seen from a comparison between the Timmins and Metro samples, that the Timmins samples contain a larger nitrogen content. Potassium and phosphorus seem to be elevated

within the AEVA units. However, it is noticeable that calcium exceeds in the Metro samples over the samples that were harvested on campus as well as from Timmins.

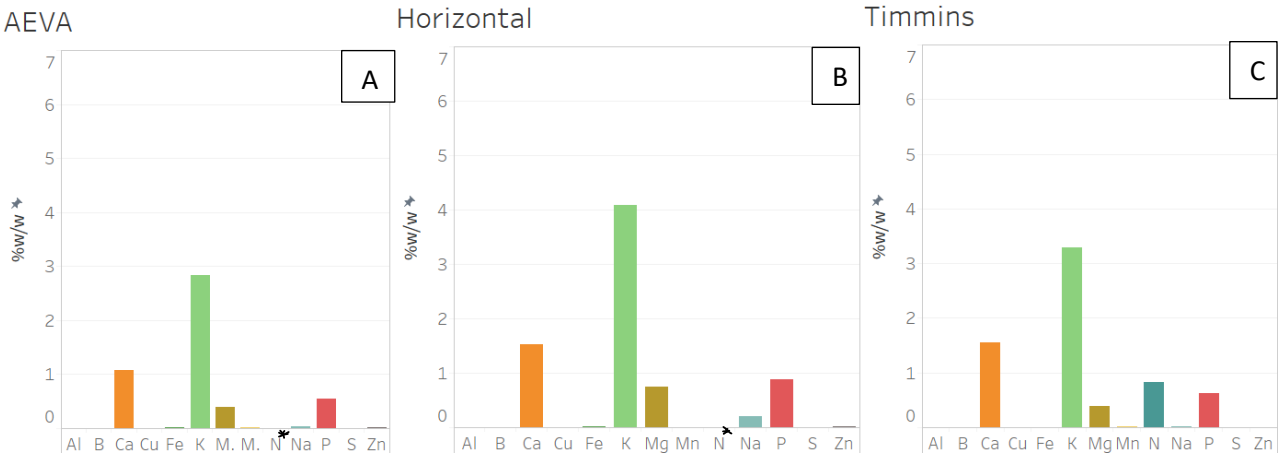


Figure 2.0: (A: AEVA, B: Horizontal & C: Timmins); Graphs A-C, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 3 times/day. Graph A and B*, neglects dictation for nitrogen content due to the fact that there was limited basil yield from the harvest. Whereas, graph C are basil samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus.

Figure 2.0 allows for the best comparison between the basil samples obtained for this specific lighting and watering parameters. At an overall glance, it is seen that the horizontal unit contained a larger concentration in the primary elements such as calcium, potassium, magnesium and phosphorus. It is also seen that there are some presence of iron and zinc as well. However, there is a larger sodium concentration in the horizontal unit than the AEVA unit and the Timmins samples. In comparison of C with Figure 1.0 D and E, there is a significant larger concentration in nitrogen content. Furthermore, in comparison of all the graphs within Figure 1.0 and Figure 2.0, it would be assumed that Figure 2.0 graph B exceeds in potassium, magnesium, sodium and phosphorus, with one of the highest concentrations of calcium as well.

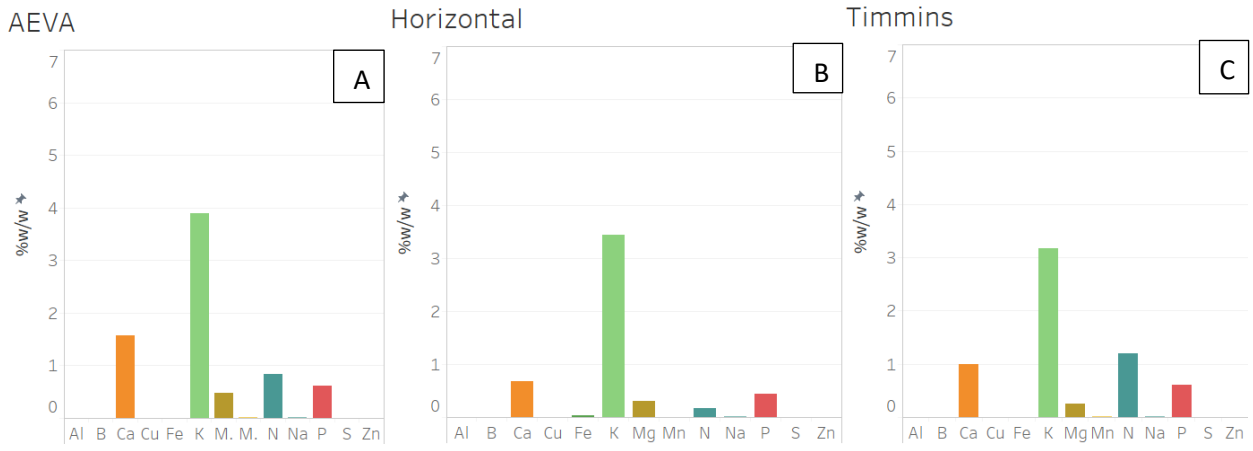


Figure 3.0: (A: AEVA, B: Horizontal & C: Timmins); Graphs A-C, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 6 times/day. Whereas, graph C are basil samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus.

Figure 3.0 acknowledges a comparison between the basil samples obtained for the specific watering and lightning regimes. Observing the graphs allows for interpretation of the basils grown upon the AEVA unit contains a larger concentration of calcium, potassium, magnesium and phosphorus. However, the horizontal unit contains a small concentration of iron. Nonetheless, the AEVA unit as well as the Timmins samples contain both manganese and sodium. Lastly, it is observed that the Timmins samples contain a larger nitrogen content compared to the AEVA and horizontal units. From Figures 1.0-3.0, it can be observed that despite all the different lighting regimes and watering cycles, grocery and Timmins; Figure 3.0 graph A, provides the greater concentration of potassium, calcium, magnesium, phosphorus and nitrogen. It also contains traces of manganese and sodium.

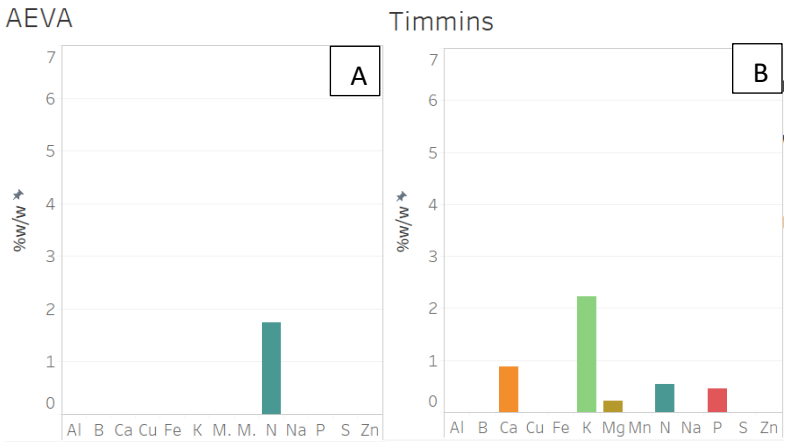


Figure 4.0: (A: AEVA & B: Timmins); Graphs A & B, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 24 times/day. Whereas, graph B are basil samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus. Graph A only contains the total nitrogen content for the basil samples as there were insufficient number of samples available to analyze for the minerals through ICP-OES.

Within Figure 4.0, it is quite noticeable that the nitrogen content within graph A exceeds in its concentration compared to all the graphs presented from Figure 1.0-3.0. However, graph A does not include the minerals from the harvest.

The most common lighting parameter is an 18/6 cycle as it is in-sync with a 24 hour/day cycle. The various watering cycles allow for various physical growth of the plants, but most importantly the amount of minerals that are absorbed within the plants, seen in Figure 5.0 – 8.0 for mint.

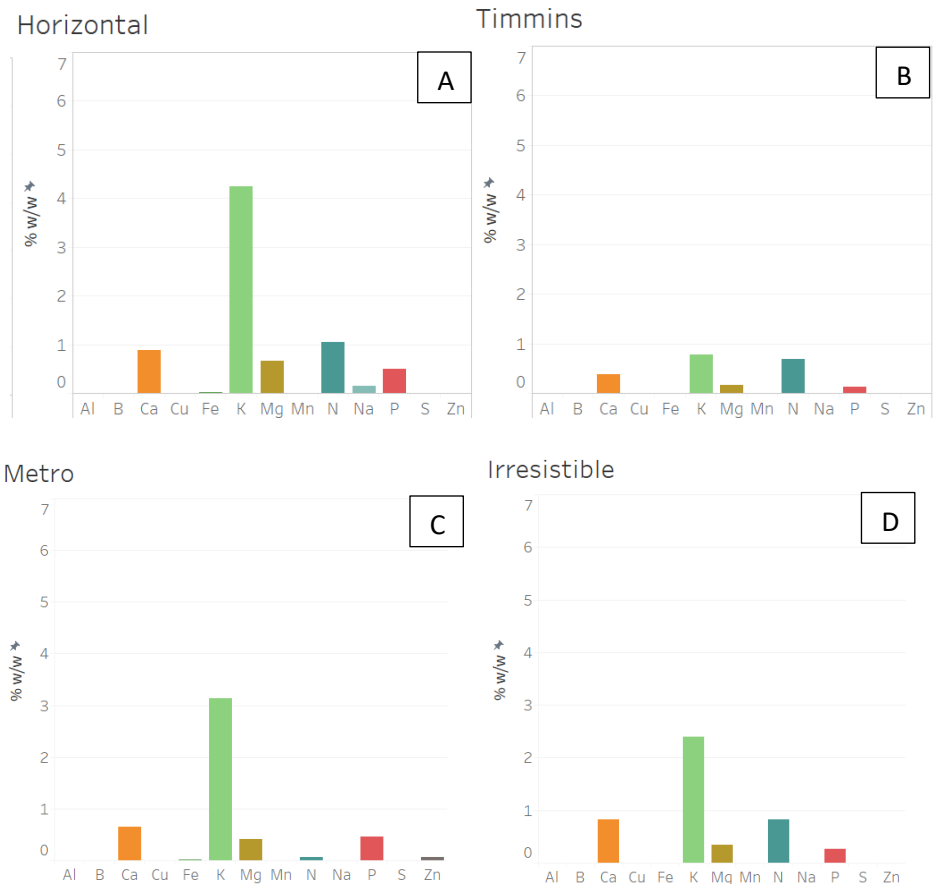


Figure 5.0: (A: Horizontal, B: Timmins, C: Metro, D: Metro – Irresistible); Graphs A & B, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 1 times/day. Whereas, graph B are mint samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus. A graph containing mint grown on the AEVA unit was not included as there were insignificant yield produced to complete ICP-OES nor nitrogen content analysis.

Figure 5.0 allows for a comparison of mint samples obtained from the horizontal units, Timmins and the Metro samples. Between the 4 graphs, it is evident that the horizontal unit contains a larger concentration in nitrogen. This varies the fact that the overall health of the plant is better when it is harvest on campus rather than having the health of the plant deplete due to travelling. The horizontal unit also exceeds in calcium, potassium, magnesium, sodium and phosphorus.

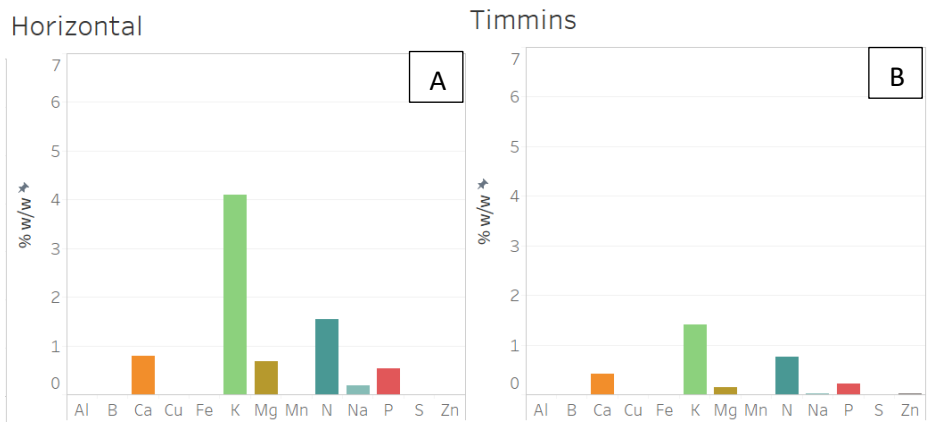


Figure 6.0:(A: Horizontal and B: Timmins); Graphs A & B, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 3 times/day. Whereas, graph B are mint samples obtained from the local farm at Timmins with the same lighting and water regimes from samples grown on campus. A graph containing the mint samples grown on the AEVA unit was not included as there were insignificant yield produced to complete ICP-OES nor nitrogen content analysis.

Figure 6.0 recognizes the correlation of the mint samples obtained from the horizontal unit and Timmins. It is clear that the horizontal unit produced a greater concentration of the minerals as well as the nitrogen content. However, it is seen that there are small but noticeable traces of zinc presence in the Timmins samples.

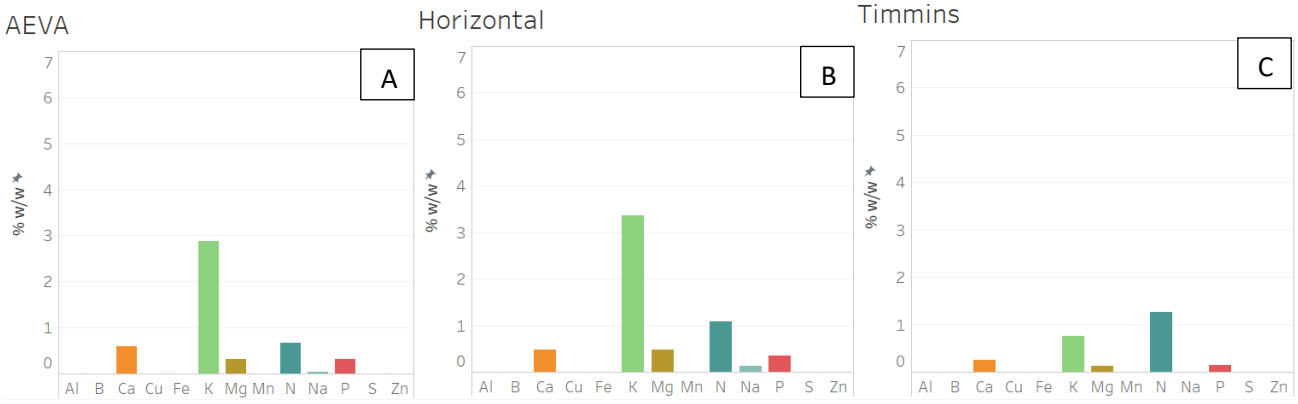


Figure 7.0:(A: AEVA, B: Horizontal and C: Timmins); Graphs A & B, presented in this figure contains a lighting regime of 18/6 with a watering cycle of 3 times/day. Whereas, graph C are mint samples obtained from the local farm at Timmins with the same lighting and water regimes from the mint samples grown on campus.

Within Figure 7.0, the samples were compared between the varying locations of growth. To one's surprise, the nitrogen content within the Timmins mint were greater than both the AEVA and horizontal units. Despite that, the horizontal unit had a larger concentration of potassium, magnesium, sodium and phosphorus. Although, the AEVA

unit contained a larger calcium concentration compared to the Timmins and horizontal samples.

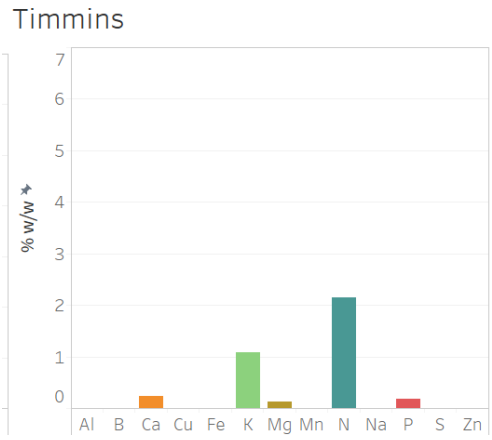


Figure 8.0: Mint grown at Timmins farm under 18/6 lighting regime with a continuous water cycle

Despite that fact that there were no samples grown on campus with identical parameters, it is an interesting observation to see the increase of nitrogen content within the samples but a significant decrease on all the other minerals. In comparison to graphs displayed in Figure 5.0-7.0, potassium generally had a larger concentration between 3-4 %w/w. Therefore, it may be looked at that, the more water the plants receive, their mineral content may become saturation until depletion of the minerals occur.

Conclusions and Implications

All samples that were analyzed for the total nitrogen content contained a dilution of 1:10 as it allowed for the most precise determination of concentration. These samples were prepared by using utilizing mass from a dry plant content that followed a procedure from the Handbook of Reference Materials for Plant Analysis, this procedure was altered and can be followed in SOP 8.2 and 8.3.

Each harvest was analyzed by ICP-OES to determine the concentration of the most common macronutrients and micronutrients, Aluminum (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Sulphur (S) and Zinc (Zn). Prior to analysis upon the ICP-OES, the harvest samples were dried, digested through the microwave digestion and diluted 1:5, which followed SOPs 8.4 and 8.5.

After analyzing all of the samples from the horizontal and vertical units, the following was concluded:

- AEVA units produced basil and mint samples with a greater concentration of the minerals, while containing a larger total nitrogen content
- From the various parameters that were analyzed, a lighting regime of 18/6 with a watering cycle of 3 times/day contained a greater yield when harvested as well as contained a greater concentration of the known mineral
- The units in comparison to the local farm in Timmins and from Metro also proved that the AEVAs contained a greater in macronutrients and micronutrients.
- From the ICP-OES analysis, concentrations for aluminum, boron, copper and zinc were all below limit of detection.
- Iron, manganese and sodium had trace amounts of content within certain parameters, but more evidently seen in the horizontal units compared to the AEVA apparatus.
- Sulphur is an important nutrient to plants as it is responsible for the uptake of minerals within the plants, however the trace amounts that may have been present within the samples are below limit of quantitation (LOQ). Therefore, although trace amounts are present, Sulphur should be looked at as a trend.
- The Sulphur trend concludes that there is more Sulphur present in mint than the basil from the horizontal units. It is also to be noted that there the concentration of Sulphur in the AEVA units are within the same range for both mint and basil. It is observed that there is a lower concentration of Sulphur present in the Timmins samples compared to the horizontal and AEVA units.

To conclude, the project was successful as it demonstrated harvests from various lighting regimes and watering cycles to optimize for a greater yield in basil and mint production as well as obtaining the most minerals within the plants.

Future Research Considerations

It would be highly recommended to analyze Sulphur under its own methodology as it provides important information in regards to the efficacy of the uptake in nutrients. Sulphur can be analyzed upon the ICP-OES to an accurate value, if Sulphur recovery was optimized from the beginning of method development to the exclusion of other elements of analysis. Specifically, the plant mass required, would have to increase and the dilution factor would decrease significantly and the instrumental parameters optimized for Sulphur recovery. With the change in these variables, there is a much higher probability of obtaining precise values for the concentration of Sulphur present in the samples.

The overall experiment had to be limited due to the mass of plant which impacted the overall results obtained. For future research, it is advised that the plants of choosing to be of a larger mass, for example kale or lettuce.

To continuously optimize the growth parameters, selecting a single lighting adjustment such as 16/2 and optimizing various water, pH and nutrimental factors are adjusted, the

best parameters for lighting of 16/2 can be optimized for the best growth of that specific plant. This same optimization can occur selecting a single watering adjustment such as 3x/day and optimizing various lighting, pH and nutrimental factors. If a plant with a larger mass is selected, once a week the pH and nutrimental factors can be adjusted and the removal of a single plug with the plant can be analyzed for its minerals. This would prove that any fluctuation of the pH or nutrients may have a drastic or mild impact upon the growth of the plant.

References

1. 1998 Miller, R.O. Extractable Nitrate in Plant Tissue: Ion-Selective Electrode Method in Handbook of Reference Methods for Plant Analysis. Ed. Yash P. Kalra. CRC Press.
2. 1997 Barnes, K.W; Debrah E., Determination of Nutrition Labeling Education Act Minerals in Foods by Inductively Coupled Plasma-Optical Emission Spectroscopy, Atomic Spectroscopy, 18, *2,* 41-54
3. 2018 Murarikova, A.; Neugebauerova, J., Seasonal variation of ascorbic acid and nitrate levels in selected basil (Ocimum basilicum L.) varieties, 45, *1,* 47-52

SOPs

8.1 Standard Operating Procedure for Total Ionic Strength Adjustment Buffer (TISAB) Solution

TITLE: Standard Operating Procedure for TISAB Solution		
Date of Preparation: January 31st, 2021	**Date of Revision:** N/A	**Revision No.:** N/A
Submitted By: Jasmin Khan	**Approved by:** N/A	**Page:** 1 of 2

1.0 PURPOSE

1.1 The purpose of this SOP is to instruct the user to prepare TISAB solution which is required for extracting Nitrate

2.0 SCOPE AND APPLICABILITY

2.1 This SOP is to be used only by the trained personnel

2.2 This SOP is to be used for the purpose of preparing an extraction solution to extract nitrate from plant samples

3.0 MATERIALS AND EQUIPMENTS

3.1 Milli Q Water
3.2 Aluminum Sulphate
3.3 Boric Acid
3.4 Sulphamic Acid
3.5 Silver Sulphate
3.6 1 L Volumetric Flask
3.7 Top Loading Balance

4.0 SAFETY PRECAUTIONS

4.1 Gloves, goggles and lab coats must be worn throughout the entire duration of preparation of the TISAB solution

5.0 PROTOCOL

5.1 Place a weigh paper on the top loading balance and tare the paper
5.2 Weigh 6.7 g of aluminum sulphate on the paper and transfer into the volumetric flask
5.3 Re-weigh the paper on the balance and record the weight

5.4 Repeat steps 5.1 – 5.3 for each reagent
- **5.4.1** Weigh 1.2 g of Boric Acid
- **5.4.2** Weigh 1 g of Sulphamic Acid
- **5.4.3** Weigh 2 g of Silver Sulphate

5.5 Fill the volumetric flask with approximately 500 – 700 mL of Milli Q Water

5.6 Place a stir bar in the volumetric flask

5.7 Place the volumetric flask on a stir plate and allow each reagent to dissolve completely

5.8 Once all the reagents have completely dissolved, top up to the mark to complete the preparation of the 1 L TISAB solution.

6.0 REFERNCES

6.1 1998 Miller, R.O. Extractable Nitrate in Plant Tissue: Ion-Selective Electrode Method in Handbook of Reference Methods for Plant Analysis. Ed. Yash P. Kalra. CRC Press.

8.2 Standard Operating Procedure for Determination of Dry Plant Content

TITLE: Standard Operating Procedure for Determination of Dry Plant Content		
Date of Preparation: January 31st, 2021	**Date of Revision:** N/A	**Revision No.:** N/A
Submitted By: Jasmin Khan	**Approved by:** N/A	**Page:** 1 of 2

1.0 PURPOSE

1.1 The purpose of this SOP is to instruct the user to dry the plants prior to analysis upon ISE and ICP-OES

2.0 SCOPE AND APPLICABILITY

2.1 This SOP is to be used only by the trained personnel

2.2 This SOP is to be used for the purpose of drying the plants prior to analysis upon ISE and ICP-OES

3.0 MATERIALS AND EQUIPMENTS

3.1 Aluminum Cups
3.2 Analytical Balance
3.3 Oven
3.4 Oven Trays
3.5 Desiccator

4.0 SAFETY PRECAUTIONS

4.1 Gloves, goggles and lab coats must be worn throughout the entire duration of preparation of drying the plants

5.0 PROTOCOL

5.1 Place the aluminum tray onto the analytical balance
5.2 Record the empty tray weight and tare the balance
5.3 Place plant sample onto the tray (mass depends upon the test method)
5.4 Record sample weight
5.5 Place the aluminum trays into the oven trays
5.6 Place the oven trays into the oven at 70°C for 2 hours
5.7 Then place the aluminum trays into a desiccator for 1 hour
5.8 Record mass of the aluminum tray

6.0 REFERENCE

6.1 1998 Miller, R.O. Extractable Nitrate in Plant Tissue: Ion-Selective Electrode Method in Handbook of Reference Methods for Plant Analysis. Ed. Yash P. Kalra. CRC Press.

8.3 Standard Operating Procedure for Determination of NO_3/N content using Ion Specific Electrode (ISE)

TITLE: Standard Operating Procedure for Determination of NO_3/N content using ISE		
Date of Preparation: January 31st, 2021	**Date of Revision:** N/A	**Revision No.:** N/A
Submitted By: Jasmin Khan	**Approved by:** N/A	**Page:** 1 of 3

1.0 PURPOSE

1.1 The purpose of this SOP is to instruct the user to operate the ISE to detect the NO_3/N content within the samples

2.0 SCOPE AND APPLICABILITY

2.1 This SOP is to be used only by the trained personnel

2.2 This SOP is to be used for the purpose of analyzing plant samples after being prepared as per protocol

3.0 MATERIALS AND EQUIPMENTS

3.1 TISAB Solution (Refer to SOP 8.1)

3.2 Milli Q Water

3.3 Vacuum Filtration

3.4 Whatman Filter No. 4

3.5 Dried Plant Samples (Refer to SOP 8.2)

3.6 Potassium Nitrate

3.7 ISE probe, membrane specific for Nitrate

4.0 SAFETY PRECAUTIONS

4.1 Gloves, goggles and lab coats must be worn throughout the entire duration of determining NO_3/N content using ISE

5.0 PROTOCOL

5.1 Prepare Calibration Curve

- **5.1.1** Prepare 1000 ppm stock of Nitrogen utilizing Potassium Nitrate
  - **5.1.1.1** Weigh 7210 mg of Potassium Nitrate
  - **5.1.1.2** Dissolve in 1 L of Milli Q Water in a volumetric flask
- **5.1.2** Complete serial dilution to have a 3 -point calibration curve
  - **5.1.2.1** 5 ppm, 20 ppm and 50 ppm

5.2 Prepare the Sample

- **5.2.1** Utilize the dry samples that were prepared as per SOP 8.2

5.2.2 Place the sample into a coffee grinder and grind
5.2.3 The outcome of the samples should be a fine coarse of less than 1 mm (similar in size to ground coffee beans)
5.2.4 Weigh 500 mg of the ground sample and place into a beaker
5.2.5 Add 25 mL of TISAB solution (refer to SOP 8.1)
5.2.6 Cover the beaker with aluminum foil
5.2.7 Place the beakers onto a mechanical shaker at 145 rpm for 30 minutes
5.2.8 Filter the sample through a vacuum filtration apparatus using a Whatman No. 4 filter paper
5.2.9 Pour the sample into a digitube
5.2.10 Rinse the filtration apparatus between every sample
5.2.11 Complete a 1:10 dilution with the sample filtrate using the TISAB solution

5.3 Analyze the standards/samples
5.3.1 Rinse the ISE probe with Milli Q Water
5.3.2 Place the 5-ppm standard in a small plastic container with a stir bar
5.3.3 Have the stir plate directly under the probe
5.3.4 Select standardization and type in the standard concentration
5.3.5 Select enter
5.3.6 Allow the standard to stir and stabilize
5.3.7 Select confirm once the mV has stabilized
5.3.8 Repeat steps 5.3.2 – 5.3.7 for each standard
5.3.9 Analyze a blank that contains TISAB solution by placing the probe into the sample with a stir bar
5.3.10 Once the concentration has stabilized record the concentration
5.3.11 Analyze all samples by pouring them individually into plastic containers with a stir bar
5.3.12 Once the concentration has stabilized record the concentration

5.4 Rinse the probe
5.5 Place probe into storage solution
5.6 Turn off ISE system

6.0 REFERENCE

6.1 1998 Miller, R.O. Extractable Nitrate in Plant Tissue: Ion-Selective Electrode Method in Handbook of Reference Methods for Plant Analysis. Ed. Yash P. Kalra. CRC Press.

8.4 Standard Operating Procedure for Extraction via Microwave Digestion

TITLE: Standard Operating Procedure for Extraction via Microwave Digestion		
Date of Preparation: January 31st, 2021	**Date of Revision:** N/A	**Revision No.:** N/A
Submitted By: Jasmin Khan	**Approved by:** N/A	**Page:** 1 of 2

1.0 PURPOSE

1.1 The purpose of this SOP is to instruct the user to extract minerals from plants through microwave digestion

2.0 SCOPE AND APPLICABILITY

2.1 This SOP is to be used only be the trained personnel
2.2 This SOP is to be used for the purpose of extraction of minerals within plants through microwave digestion prior to analysis upon ICP- OES

3.0 MATERIALS AND EQUIPMENTS

3.1 Concentrated Nitric Acid (70% Trace Metal Grade)
3.2 Concentrated Hydrochloric acid
3.3 3% Hydrogen Peroxide
3.4 Microwave Tubes
3.5 Microwave Digestion Unit

4.0 SAFETY AND PRECAUTIONS

4.1 Gloves, goggles and lab coats must be worn throughout the entire duration of preparing the samples

5.0 PROTOCOL

5.1 Turn on the microwave
5.2 Add 250 mg of plant sample to each microwave tube
5.3 Add 7 mL of concentrated nitric acid
5.4 Add 2 mL of concentrated hydrochloric acid
5.5 Add 1 mL of 3% hydrogen peroxide
5.6 Allow the reagents and plant sample to pre-digest for 15 minutes
5.7 Cap the tubes tightly and place in the microwave tray
5.8 Place microwave tray into the microwave digestion unit
5.9 Create the digestion method (completed once and saved for future digestions)

- **5.9.1** Stage 1
  - **5.9.1.1** Target Temperature: 160°C
  - **5.9.1.2** Ramp: 5 minutes
  - **5.9.1.3** Hold Time: 5 minutes
  - **5.9.1.4** Power: 90 W
- **5.9.2** Stage 2:
  - **5.9.2.1** Target Temperature: 190°C
  - **5.9.2.2** Ramp: 5 minutes
  - **5.9.2.3** Hold Time: 20 minutes
  - **5.9.2.4** Power: 100 W
- **5.9.3** Stage 3:
  - **5.9.3.1** Target Temperature: 50°C
  - **5.9.3.2** Ramp: 1 minutes
  - **5.9.3.3** Hold Time: 30 minutes
  - **5.9.3.4** Power: 0 W

5.10 Click Start

5.11 Once the digestion has completed, remove the tray and place in a fumehood

5.12 Remove the cap from the microwave tube, turning the cap slowly and away from the user to allow the expelled gas to be vented

5.13 Pour the content into a beaker

5.14 Completed steps 5.12 – 5.13 for each microwave tube

5.15 Add 5 mL of digested sample into a 25 mL volumetric flask

5.16 Add 500 uL of 100 ppm Y ISTD into the volumetric flask

5.17 Dilute the samples with 2% nitric acid

8.5 Standard Operating Procedure for Operating the ICP-OES

TITLE: Standard Operating Procedure for Operating the ICP-OES		
Date of Preparation: January 31st, 2021	**Date of Revision:** N/A	**Revision No.:** N/A
Submitted By: Jasmin Khan	**Approved by:** N/A	**Page:** 1 of 3

1.0 PURPOSE

1.1 The purpose of this SOP is to instruct the user to operate the ICP – OES used to ran the samples

2.0 SCOPE AND APPLICABILITY

2.1 This SOP is to be used only by the trained personnel

2.2 This SOP is to be used for the purpose of running plant samples extracted as per protocol

3.0 MATERIALS AND EQUIPMENTS

3.1 Milli Q Water

3.2 2% Nitric Acid ICP Grade

3.3 Wavelength Calibration Solution

3.4 250 mL Erlenmeyer flask

4.0 SAFETY PRECAUTIONS

4.1 Gloves, goggles and lab coats must be worn throughout the entire duration of operating the ICP-OES

5.0 PROTOCOL

5.1 Turn on all components of the ICP including the computer and cooler

5.2 Log onto the computer and open the ICP software

5.3 Create a new method

5.4 Select the internal standard element and label it as internal standard

5.5 Select all other elements that will be tested (these elements should be present within the standard)

5.6 Enter the concentration of each element in the standard and indicate it in the corresponding box so that the software knows what to look for

5.7 Set all parameters for the ICP plasma

- **5.7.1** Power: 1.35 kW
- **5.7.2** Plasma Flow: 15.0 L/min
- **5.7.3** Auxiliary Flow: 1.50 L/min
- **5.7.4** Nebulizer Pressure: 210 kPa

- **5.7.5** Viewing Height: 10 mm
- **5.7.6** Replication Read Time: 5 s
- **5.7.7** Stabilization Delay: 17 s
- **5.7.8** Sample Uptake Delay: 20 s
- **5.7.9** Pump Rate: 15 rpm
- **5.7.10** Rinse Time: 12 s
- **5.7.11** Replicates: 3

5.8 Select appropriate wavelength for each element

- **5.8.1** Aluminum → 396.152 nm
- **5.8.2** Boron → 249.772 nm
- **5.8.3** Calcium → 396.847 nm
- **5.8.4** Copper → 327.395 nm
- **5.8.5** Iron → 238.204 nm
- **5.8.6** Potassium → 766.491 nm
- **5.8.7** Magnesium → 279.553 nm
- **5.8.8** Manganese → 257.610 nm
- **5.8.9** Sodium → 589.592
- **5.8.10** Phosphorus → 213.618 nm
- **5.8.11** Sulphur → 181.972 nm
- **5.8.12** Zinc → 213.857 nm

5.9 Enter each sample name

- **5.9.1** Blank
- **5.9.2** Standards (multiple)
- **5.9.3** Blank with ISTD
- **5.9.4** Sample Blank x 2 (digested in microwave)
- **5.9.5** Samples
- **5.9.6** Blank with ISTD
- **5.9.7** 2 standards (to ensure non-minimum fluctuation/ assess percent recovery)

5.10 Turn the gas on and light the torch

5.11 With the wavelength calibration solution:

- **5.11.1** Calibrate the wavelength
- **5.11.2** Calibrate the detector
- **5.11.3** Store the data

5.12 Put the tubing of the ICP into the 2% Nitric

5.13 Press play and then the instrument will ask for the corresponding sample

5.14 Feed the tube into the sample and select enter

5.15 Repeat steps 5.12-5.13 until all samples, standards and blanks have been completed

5.16 Turn the torch off once the run has completed

5.17 Ensure all the curve are present for every element in each sample

5.18 Save the data and print the analysis

5.19 Turn the instrument off

5.19.1 Turn off each component, including the cooler and computer.

Appendix

Kale Samples

Over the past few year, Kale has become a globally known vegetable and a superfood. Despite containing an abundant source of proteins, vitamins and antioxidants, many minerals such as Calcium, Potassium, Manganese, and Magnesium are also present. Therefore, one would want to include kale in a part of their healthy diet as it contains many essential nutrients.

As a part of the Just Vertical collaboration with Seneca Innovation, Kale samples that were grew on farms in Timmins were compared to Kale samples bought at Metro. This comparison would allow for a deeper dive into the nutritional benefits of freshly grown kale against kale from the grocery stores.

It is important to note that the species of Kale from Timmins as well as from Metro are Starbor Kale – native to New Zealand. The overall purpose of this experiment is to compare mass production of farming with kale against kale growing within a vertical hydroponic unit.

Table 7.0: Kale Samples Analyzed from ICP-OES and NO3/N to Obtain Mineral Analysis in % w/w

Sample	Ca	K	Mg	Mn	Na	P	S	N
Primary Farm	0.87	2.32	0.43	0.004	0.01	0.32	0.0028	0.071
Macro Farm	0.97	2.04	0.57	0.005	0.02	0.26	0.0036	0.18
Metro	0.94	1.75	0.14	0.001	0.08	0.24	0.0028	0.0087
Organic Kale Metro	1.94	1.48	0.15	0.001	0.06	0.12	0.0042	0.024
Organic Baby Kale	1.74	2.05	0.26	0.0024	0.43	0.31	0.0043	0.08

Multiple elements, including Aluminum, Boron, Calcium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, Sulphur and Zinc were selected at when analyzing the kale samples through ICP-OES. However, the elements that contained concentrations that were above the limit of detection are foreseen in Table 7.0, these include Calcium (Ca), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P) and Sulphur (S), including Nitrogen (N) from the ISE analysis.

Note: Despite the results for organic baby kale being included in Table 8.0, the data is not compared to any of the other kale samples findings, due to the difference in maturity of the samples.

Calcium

The concentrations of Calcium for the kale samples from the hydroponic farms are relatively within the same range of the Metro kale sample. However, the organic kale sample from Metro has a surprisingly larger concentration of approximately 3x more calcium than the farms and Metro sample.

Potassium

Potassium is one of the most important minerals for a human's body as it helps with muscles and nerves. Kale is also a great source of potassium and from the analysis completed upon the ICP – OES, it can be concluded that hydroponically grown kale contain a higher source of potassium than in Metro samples, including organic kale. It was determined that the hydroponic farms contain approximately 2% w/w of potassium whereas the metro samples contain approximately 1.5% w/w of potassium.

Magnesium

Magnesium is a beneficial mineral as it helps with biochemical reactions throughout the body. It is evident through the analysis that the hydroponic farms kale contains a higher source of magnesium of approximately 3x, compared to metro kale samples, including the organic sample. The daily dose of Magnesium that adults should intake is 0.4% w/w, with the farm samples, there is 0.4 -0.5% w/w, whereas the metro samples contain 0.15% w/w.

Manganese

Manganese is a mineral that is often forgotten as a large daily dose of it, is not required. However, it is also an important mineral as it helps regulate body function. Ultimately the kale samples from the farms are 5x more nutritious compared to the metro samples.

Sodium

From looking at Table 7.0, It can be concluded that the kale samples gathered from Metro contain a higher concentration of sodium at 0.06 – 0.08% w/w. Whereas, the farm kale samples contain 0.01 – 0.02% w/w.

Phosphorus

Phosphorus is an essential mineral for bone and teeth growth, and from the ICP-OES analyze it seems that the primary farm kale has a larger concentration of phosphorus with 0.32% w/w. It is also to be noted that the macro farm sample as well as the Metro sample contain relatively the same concentration of 0.24 – 0.26% w/w. Surprisingly, the organic kale from metro has the least amount of phosphorus present with 0.12% w/w.

Sulphur

The concentrations for Sulphur are highlighted in blue to signify that the values are not precise however the trend concludes that the primary farm and the metro samples show little to no variation in their concentrations. As well as the macro farm and organic kale metro samples as well shows little variation in their concentration. To conclude for the Sulphur analysis, it would be stated that the trend is strong enough to proclaim that there is not much difference between Metro Kale and the Kale from the farms that were hydroponically grown.

Nitrogen

Nitrogen is an essential element as it allows one to determine the overall health of a plant for a qualitative aspect rather than a physical observation. It is very easy to look at plants and observe the overall health by the leaves, roots and colour. However, an analytical perspective helps determine the nitrogen content through analytical testing. Ion Specific Electrode was the method of choice to determine nitrogen content within the kale samples. All the samples have a low nitrogen content, but the macro and primary farms' kales contain a slightly higher concentration of nitrogen. It is also important to note that this batch of kale samples were frozen at -85°C, for roughly 2 months prior to analysis. With that in mind, the Macro farm's kale contained the highest nitrogen content.

Furthermore, it should be concluded that the minimal amount of analysis completed upon the comparison of the two different types of kale samples, shows that ultimately hydroponically grown farm samples have better nutrimental factors that are more beneficial to the human nutritional diet.

Pictures Throughout Project



Figure 9.0: Samples shaking on mechanical shaker

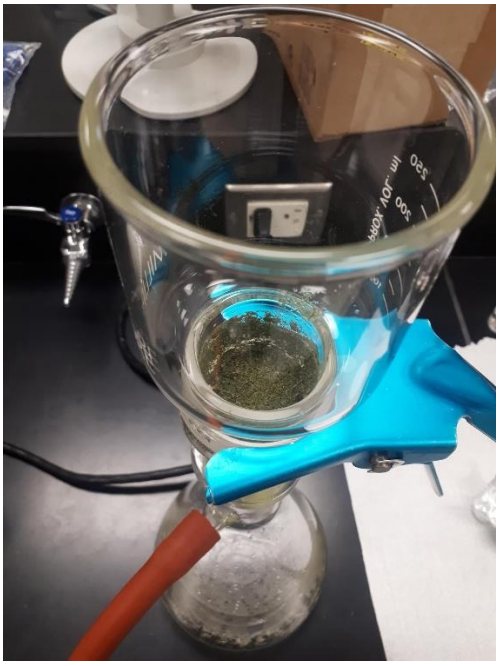


Figure 10.0: Samples being filter via vacuum filtration

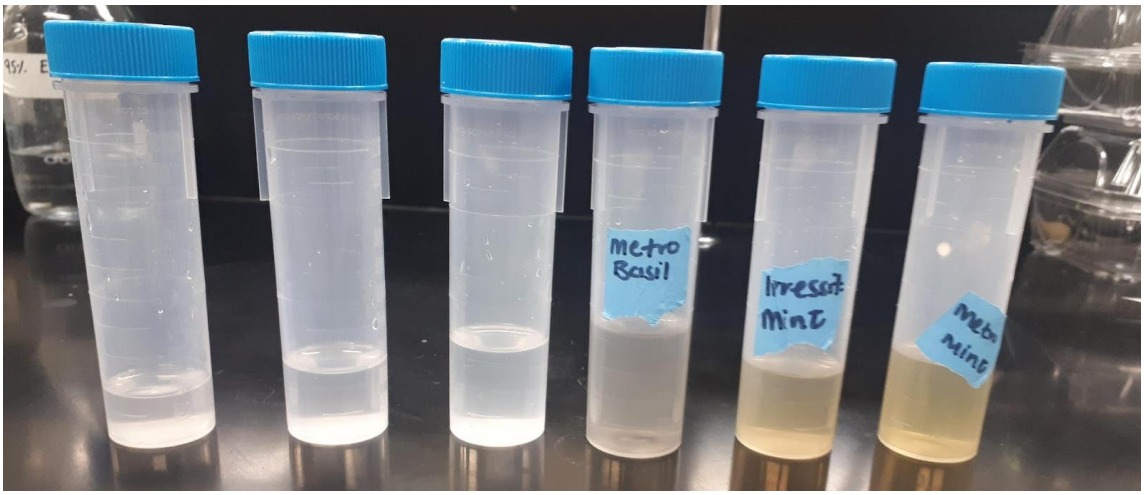


Figure 11.0: ISE raw sample comparison between AEVA harvest against grocery samples (viewed left to right: AEVA 2 basil, AEVA 1 mint, horizontal top shelf basil, Metro basil, Irresistible mint, Metro mint)

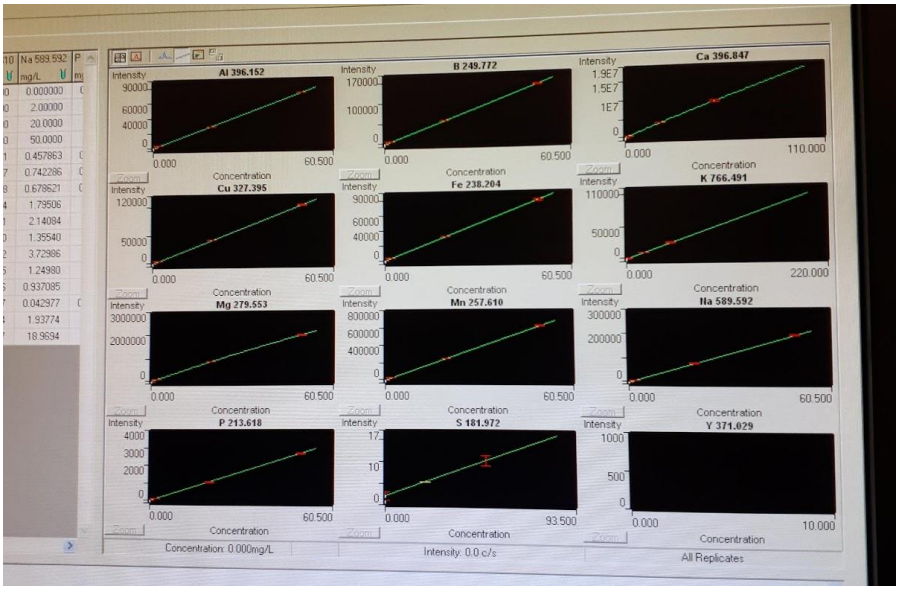


Figure 12.0: Calibration curve of sample blank from ICP-OES

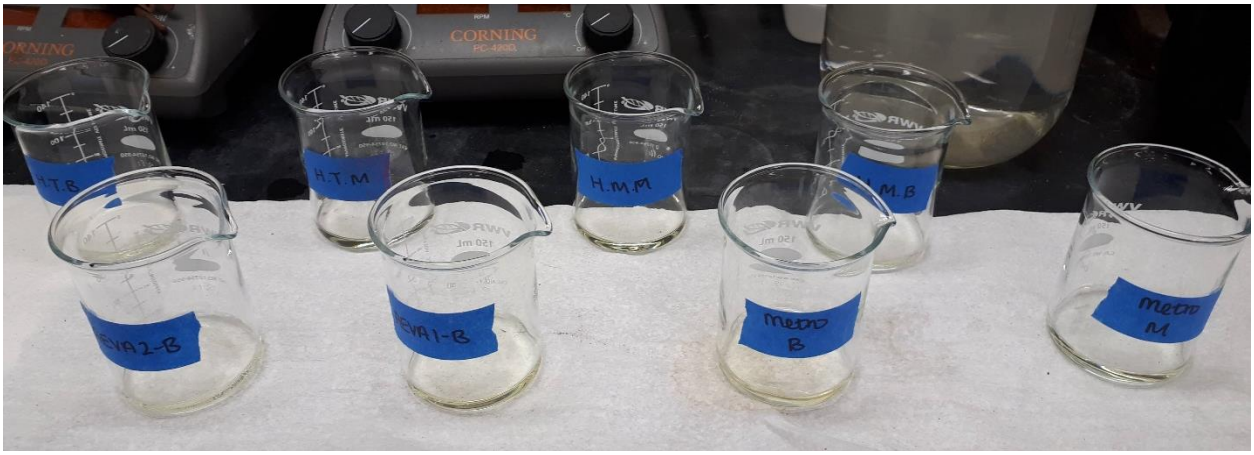


Figure 13.0: Samples removed from microwave digestor prior to dilution for ICP-OES analysis

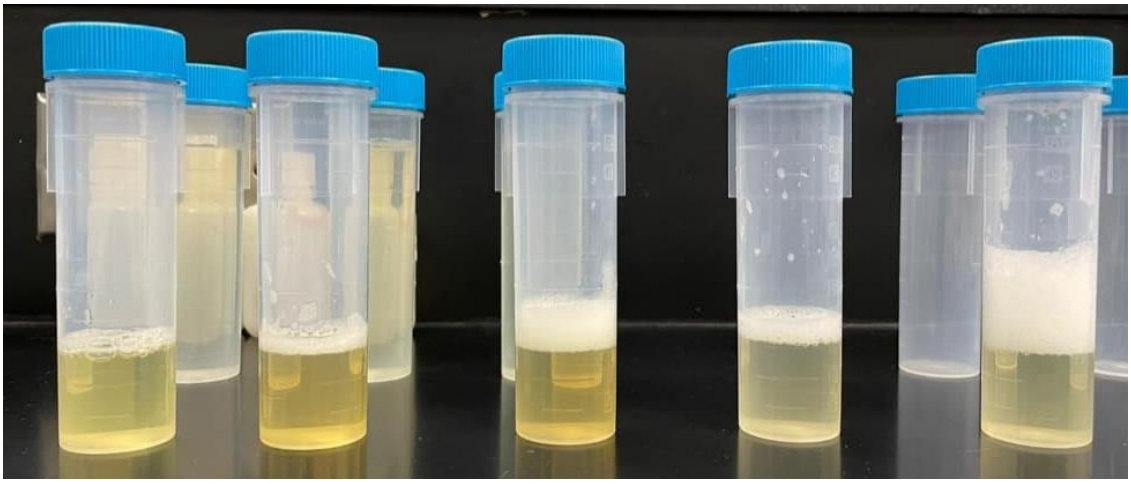


Figure 14.0: Kale samples obtained and filtered prior to ISE analysis (viewed left to right: metro, baby organic kale, organic metro, primary farm, macro farm).

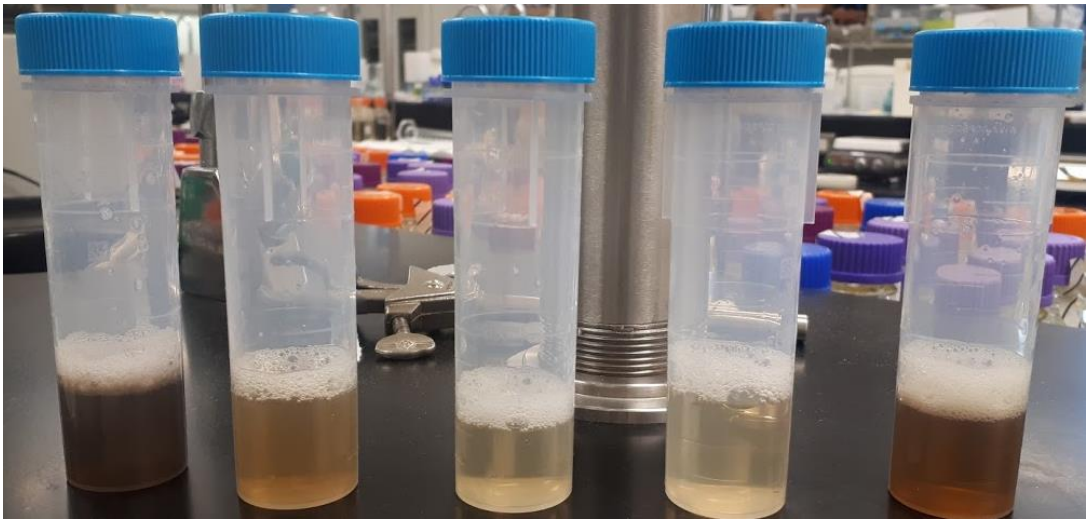


Figure 15.0: Kale samples obtained and filtered prior to ISE analysis but left for a week (viewed left to right: metro, organic metro, macro farm, primary farm, baby kale).



Figure 16.0: Yttrium used as the internal standard for ICP-OES

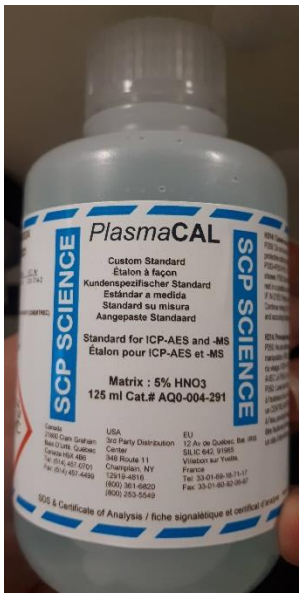


Figure 17.0: Customized standard ordered containing Aluminum (Al), Boron (B), Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Sulphur (S) and Zinc (Zn) for ICP-OES.

Graphs for the various watering cycles and its lighting regime

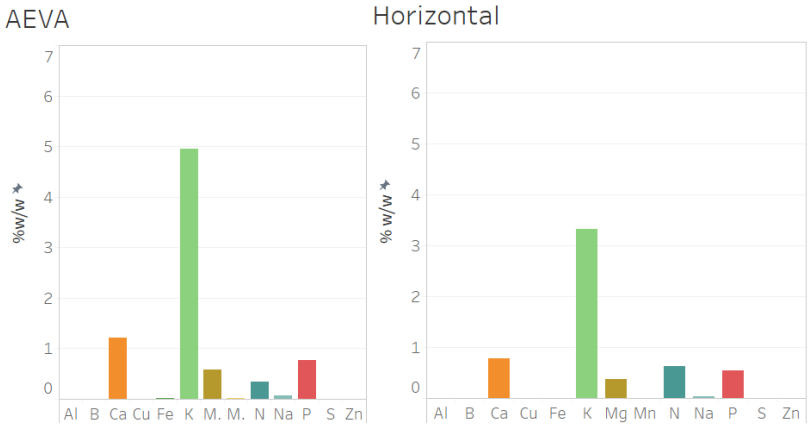


Figure 18.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle 1 time/day.

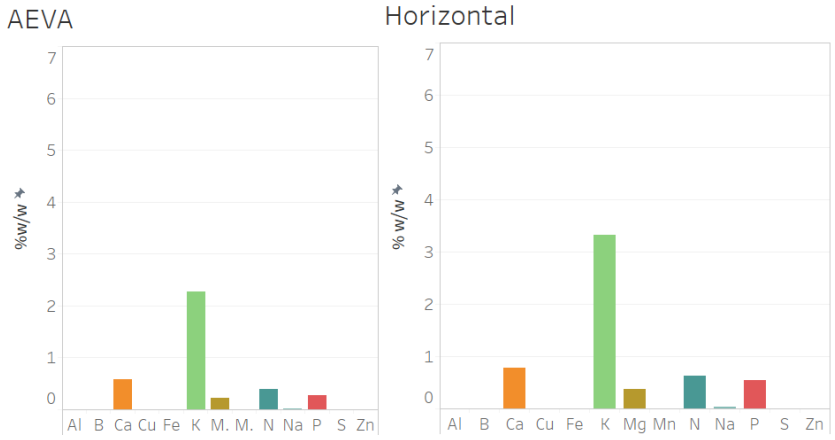


Figure 19.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle of 3 times/day.

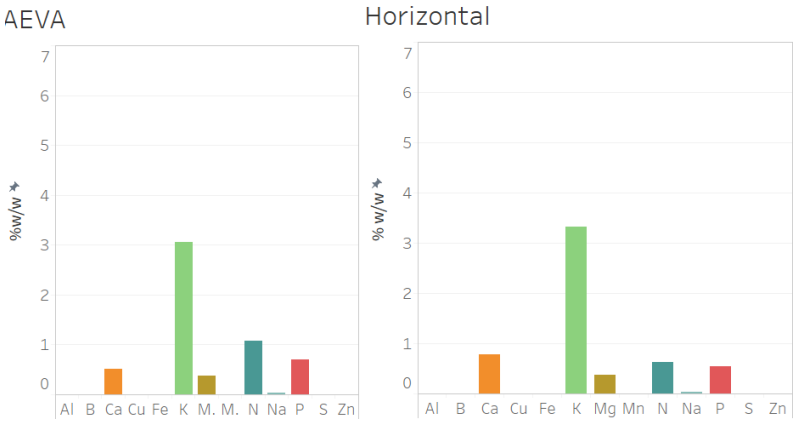


Figure 20.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle of 6 times/day.

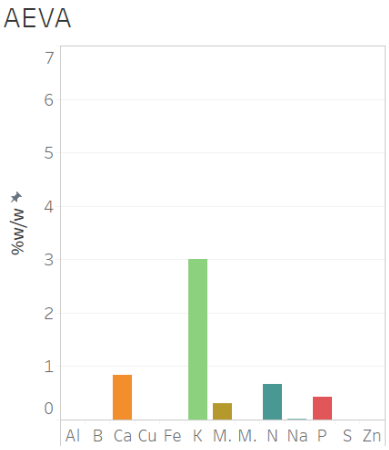


Figure 21.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a continuous watering cycle.

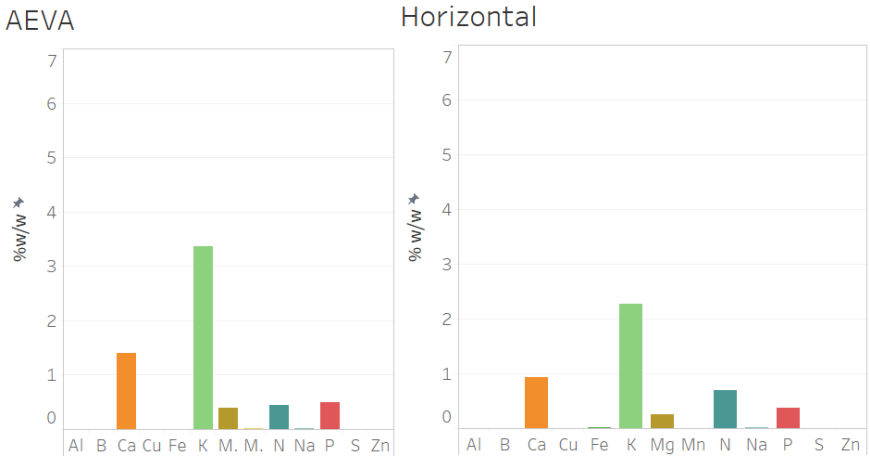


Figure 22.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 1 time/day.

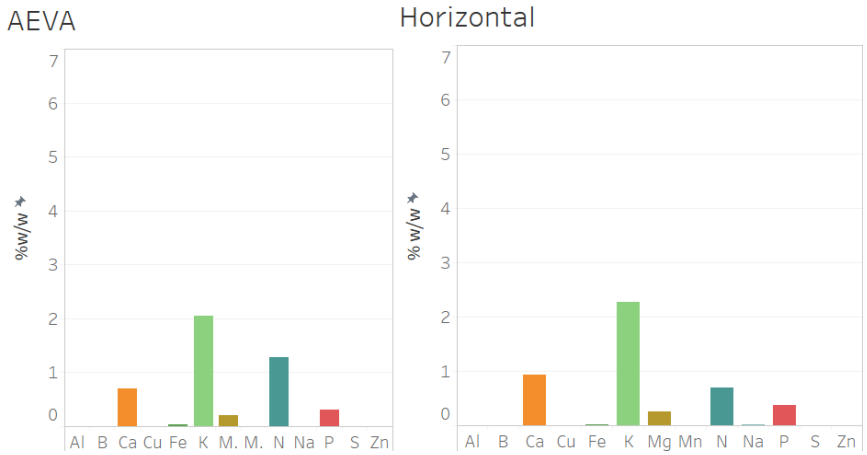


Figure 23.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 3 times/day.

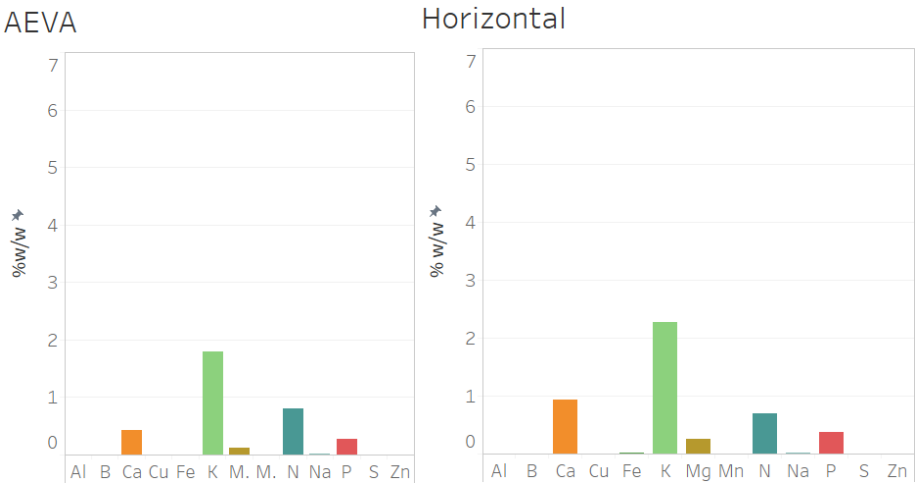


Figure 24.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 6 times/day.

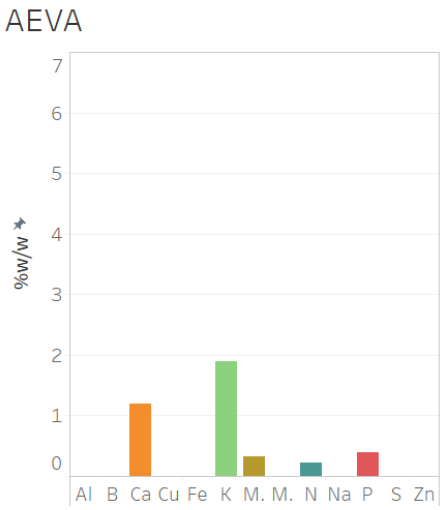


Figure 25.0: Basil samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a continuous watering cycle.

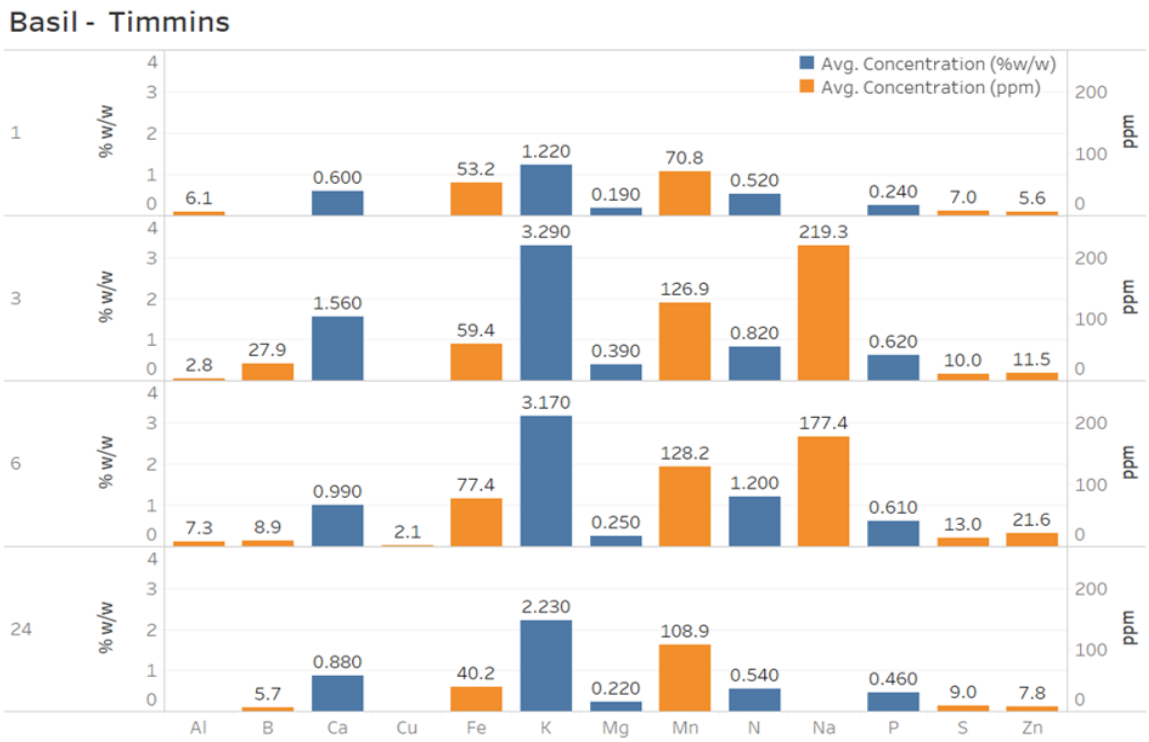


Figure 26.0: Basil samples received from Timmins to compare the various watering cycles

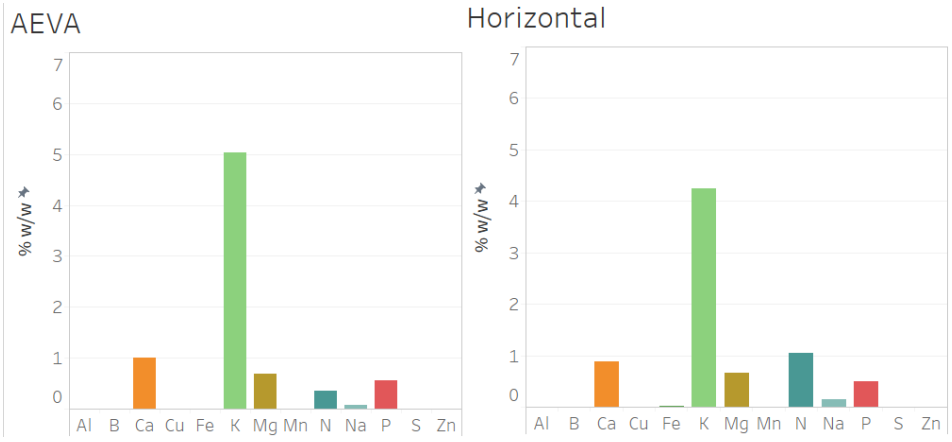


Figure 27.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle of 1 time/day.

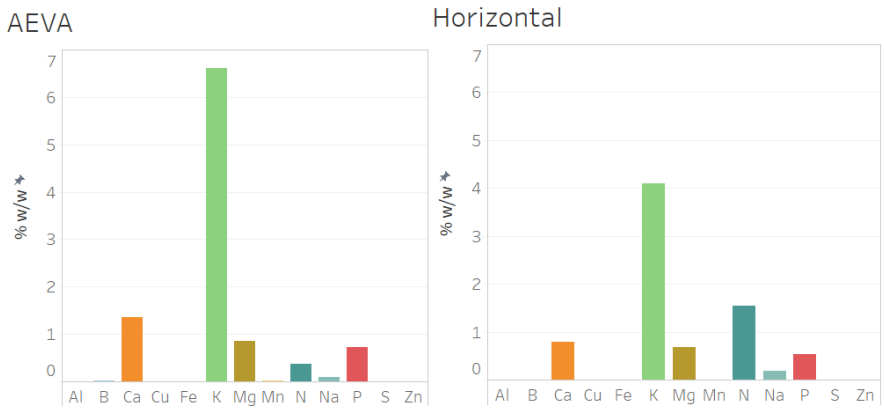


Figure 28.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle of 3 times/day.

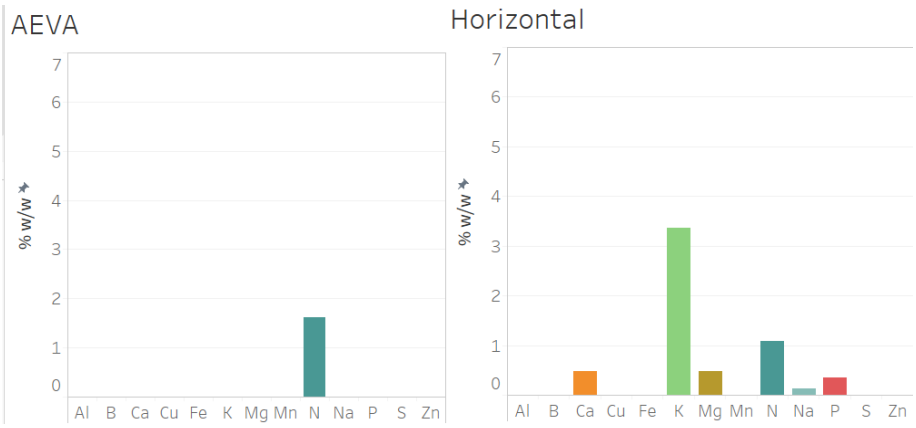


Figure 29.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a watering cycle of 6 times/day.

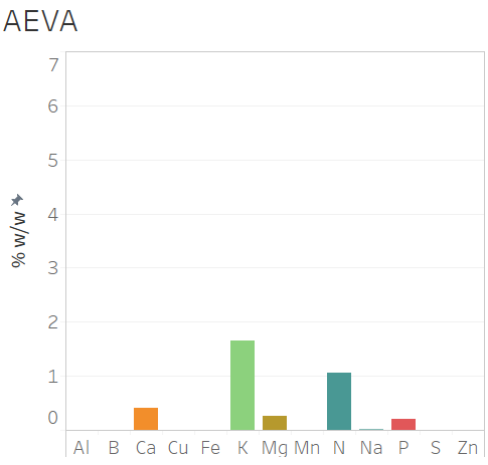


Figure 30.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 16/2 and a continuous watering cycle.

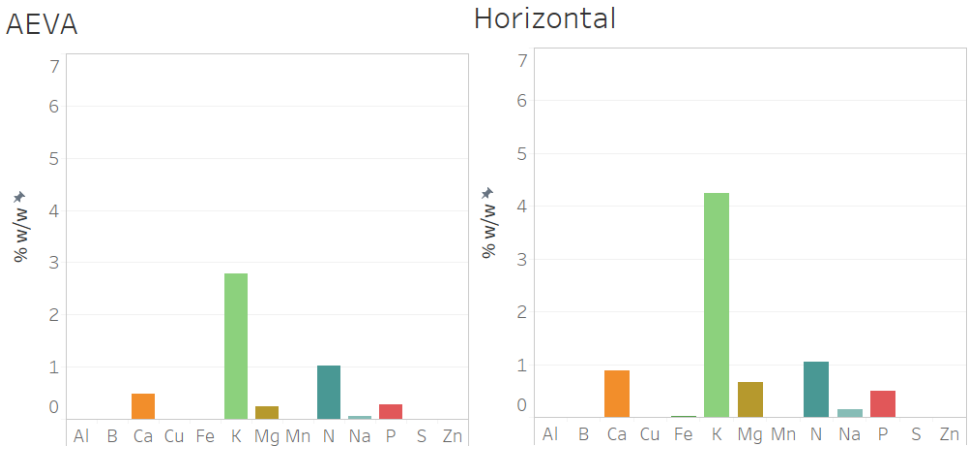


Figure 31.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 1 time/day.

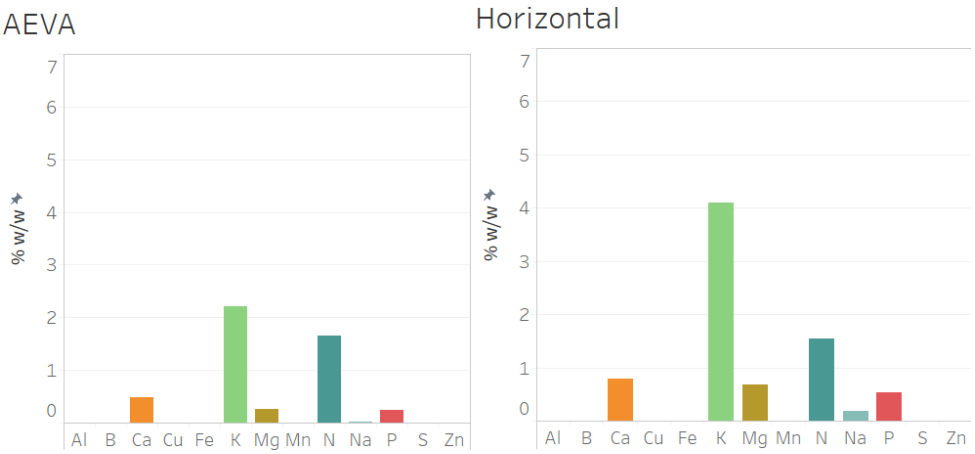


Figure 32.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 3 times/day.

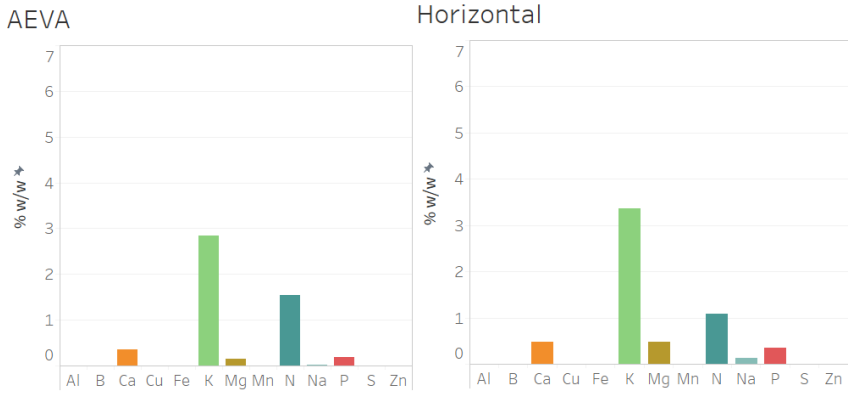


Figure 33.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a watering cycle of 6 times/day.

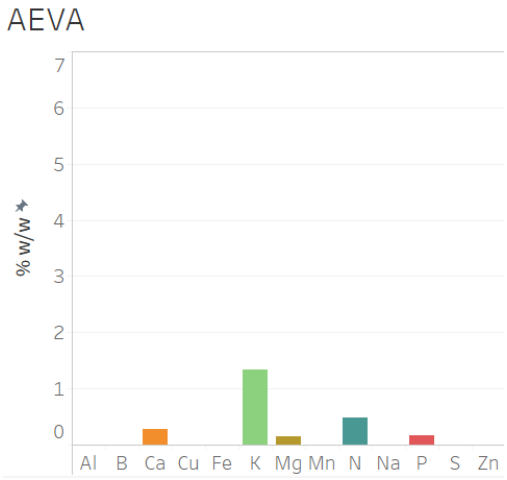


Figure 34.0: Mint samples grown on campus within the AEVA and horizontal units under a lighting regime of 24/0 and a continuous watering cycle.

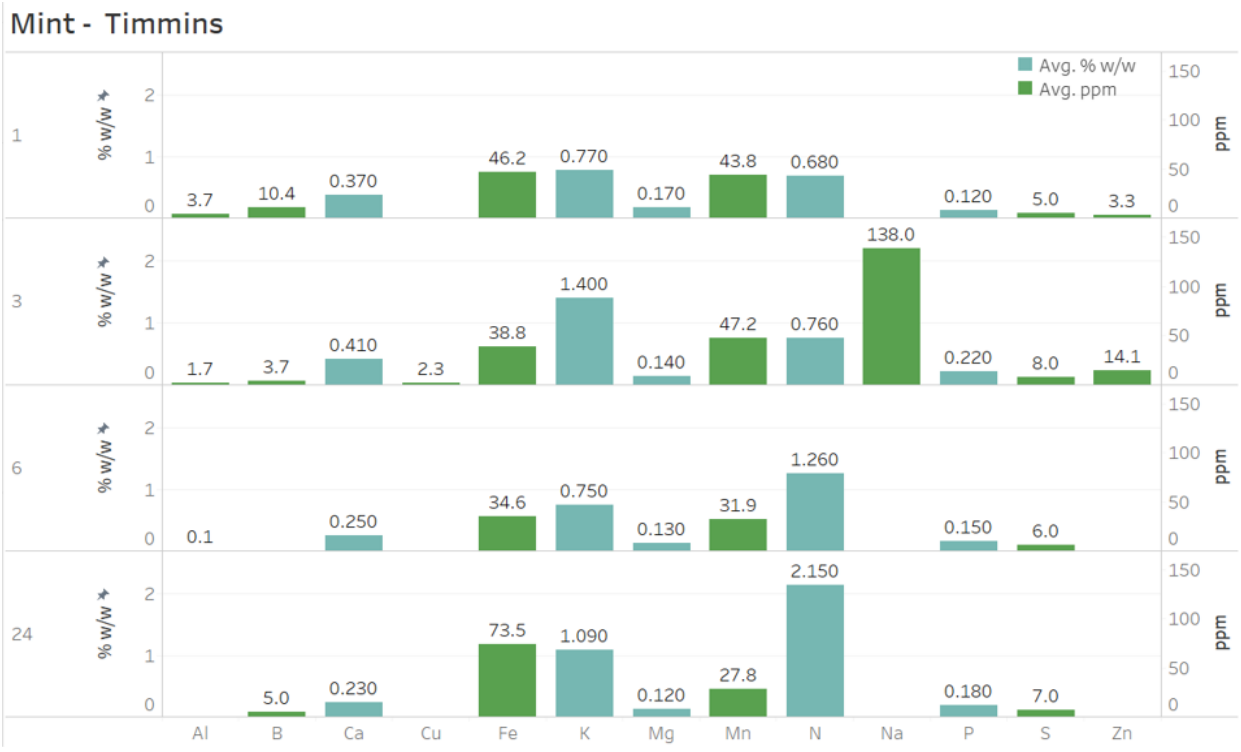


Figure 35.0: Mint samples received from Timmins to compare all the watering cycles.

Figures and Tables to compare pH and EC for each watering cycle and lighting regime within the various units

Table 8.0: Physical parameters as well comparing pH and EC for mint samples grown and harvested from the AEVA

AEVA's Mint

Watering Times	Light Cycles	pH	EC	avg. height	avg. Weight	avg. root weight
1x	18/6	5.6	1.6	13.9	0.3	8.6
	16/2	5.4	1.5	13.8	0.8	7.2
	24/0	5.3	1.6	14.2	1.2	6.5
3x	18/6	5.4	1.6	10.5	0.3	9.7
	16/2	5.4	1.5	13.8	0.8	7.2
	24/0	6.0	1.3	12.9	0.5	7.4
6x	18/6	5.2	1.5	14.7	7.4	1.4
	16/2	5.3	1.5	4.0	0.1	9.3
	24/0	5.0	1.6	11.9	0.9	8.9
cts	18/6	5.5	1.5	6.0	0.1	9.3
	16/2	5.3	1.6	22.8	2.5	10.7
	24/0	5.7	1.6	19.5	4.0	11.6

Table 9.0: Physical parameters as well comparing pH and EC for basil samples grown and harvested from the AEVA

AEVA's Basil

Number of Cycles	Light Cycles	pH	EC (mS/cm)	Plant Avg Height (cm)	Plant Avg Weight (g)	Avg. Root Weight (g)
1x	18/6	5.6	1.6	10.1	2.5	7.7
	16/2	5.4	1.5	6.1	0.7	6.8
	24/0	5.3	1.6	9.3	2.3	7.0
3x	18/6	5.4	1.6	11	3.5	9.7
	16/2	5.4	1.5	6.1	0.7	7.2
	24/0	6.0	1.3	10.2	1.8	7.4
6x	18/6	5.2	1.5	17.9	5.5	1.4
	16/2	5.3	1.5	5.5	0.9	9.3
	24/0	5	1.6	14.7	3.7	8.9
cts	18/6	5.5	1.5	7.5	1	9.3
	16/2	5.3	1.6	18.8	6.3	10.7
	24/0	5.7	1.6	20	9.5	11.6

Table 10.0: Physical parameters as well comparing pH and EC for mint samples grown and harvested from the horizontal unit

Horizontal Mint

Watering Times	Light Cycles	pH	EC	avg. height	avg. Weight	avg. root weight
1x	18/6	5.3	1.6	11.0	0.5	9.0
	16/2	5.2	1.5	11.1	0.5	7.9
	24/0	5.2	1.6	11.4	1.1	7.8
3x	18/6	5.4	1.6	11.7	0.7	9.5
	16/2	5.8	1.5	9.7	0.5	7.8
	24/0	5.5	1.3	11.1	0.6	8.1
6x	18/6	5.4	1.5	12.9	1.1	9.9
	16/2	5.2	1.5	10.5	0.7	7.9
	24/0	5.4	1.6	14.2	1.2	6.5
cts	18/6	-	-	-	-	-
	16/2	-	-	-	-	-
	24/0	-	-	-	-	-

Table 11.0: Physical parameters as well comparing pH and EC for basil samples grown and harvested from the horizontal

Horizontal Basil

Number of Cycles	Light Cycles	pH	EC (mS/cm)	Plant Avg Height (cm)	Plant Avg Weight (g)	Avg. Root Weight (g)
1x	18/6	5.3	1.6	7.9	1.2	8.2
	16/2	5.2	1.5	5.5	0.7	8.2
	24/0	5.2	1.6	9.5	1.4	7.8
3x	18/6	5.4	1.6	18.2	4.2	10.2
	16/2	5.8	1.5	7.8	1.7	7.8
	24/0	5.5	1.3	17.0	3.9	8.3
6x	18/6	5.4	1.5	14.2	2.3	10.1
	16/2	5.2	1.5	9.6	1.6	8.2
	24/0	5.4	1.6	9.3	2.3	7
cts	18/6	-	-	-	-	-
	16/2	-	-	-	-	-
	24/0	-	-	-	-	-

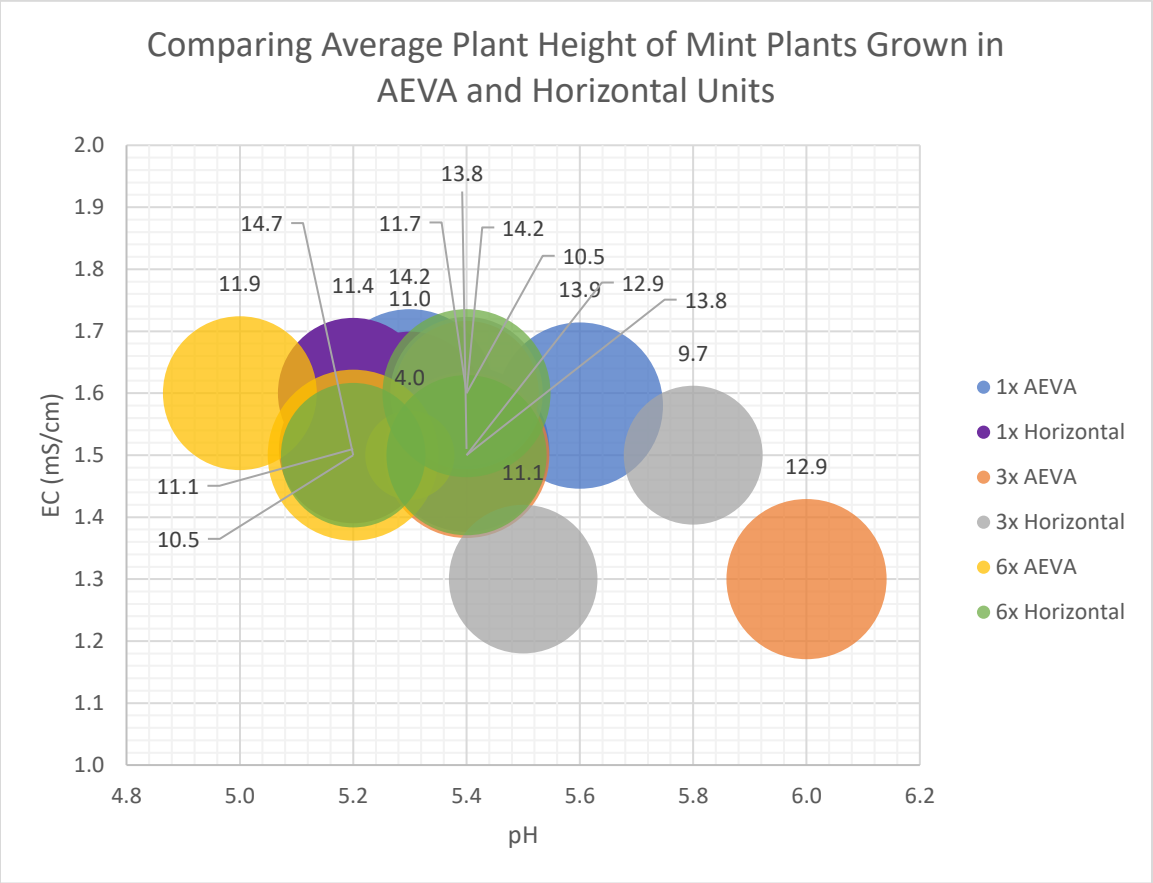


Figure 36.0: Comparison of the average plant height of mint plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Mint samples grown on the AEVA with a watering cycle of 1 times/day at a lighting regime of 24/0 shown the best overall growth.

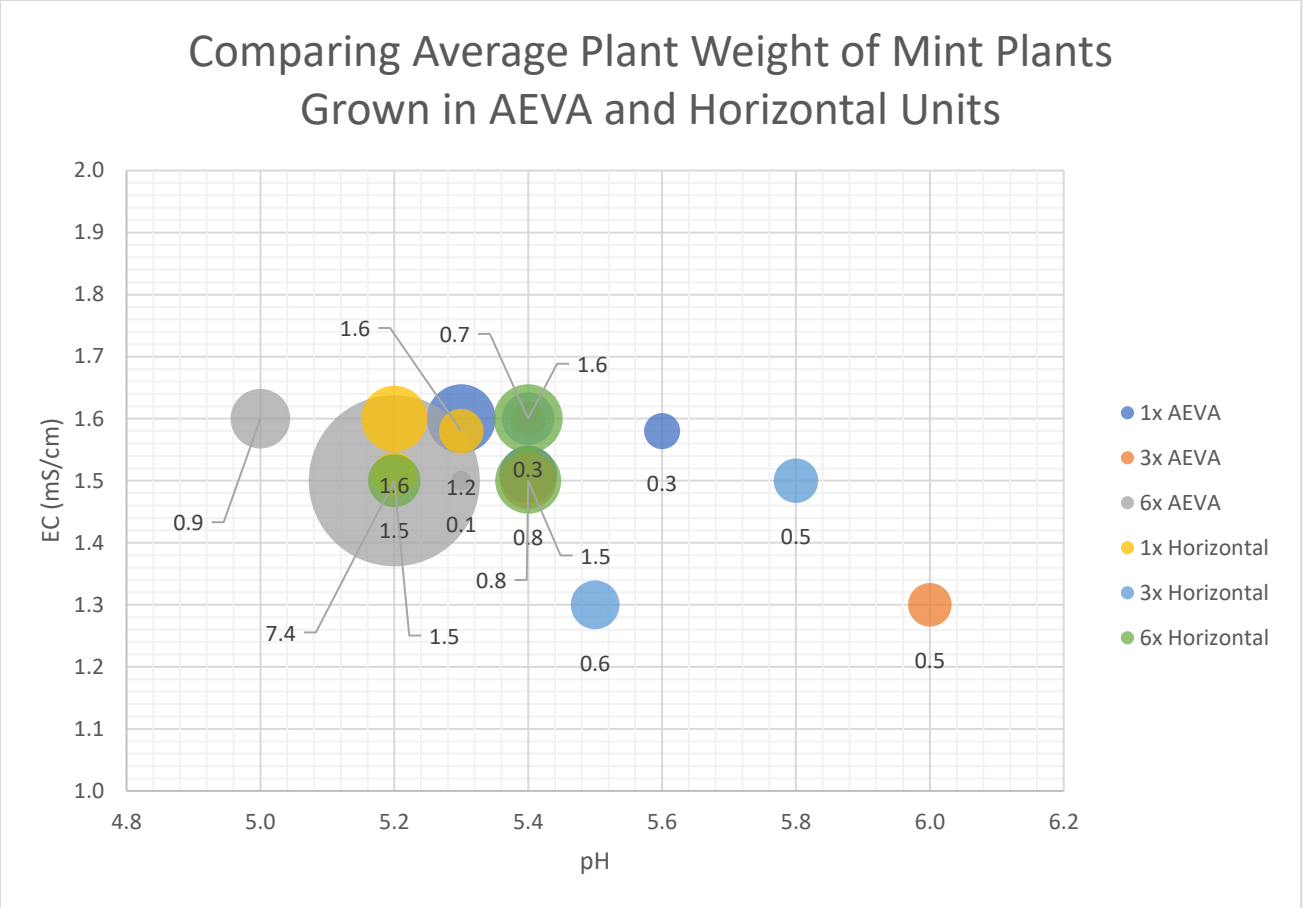


Figure 37.0: Comparison of the average plant weight of mint plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Mint samples grown on the AEVA with a watering cycle of 6 times/day at a lighting regime of 18/6 shown the best overall growth.

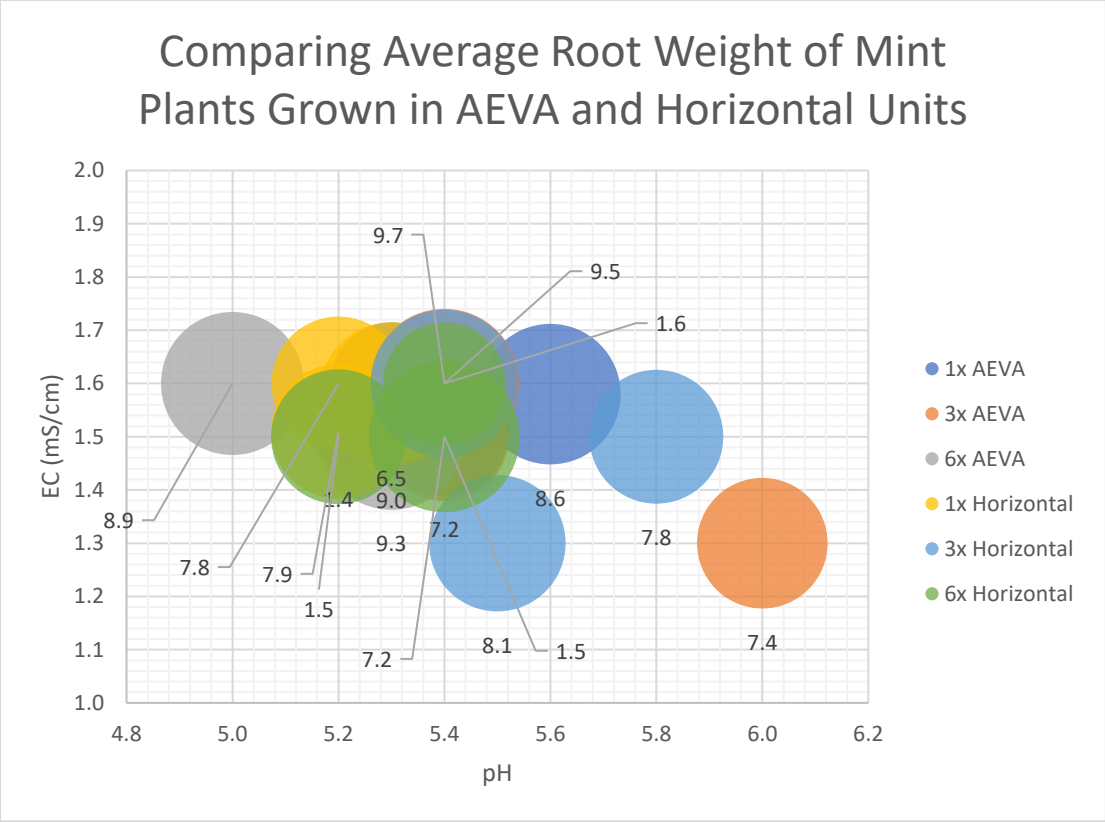


Figure 38.0: Comparison of the average root weight of mint plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Mint samples grown on the AEVA with a watering cycle of 3 times/day at a lighting regime of 18/6 shown the best overall growth.

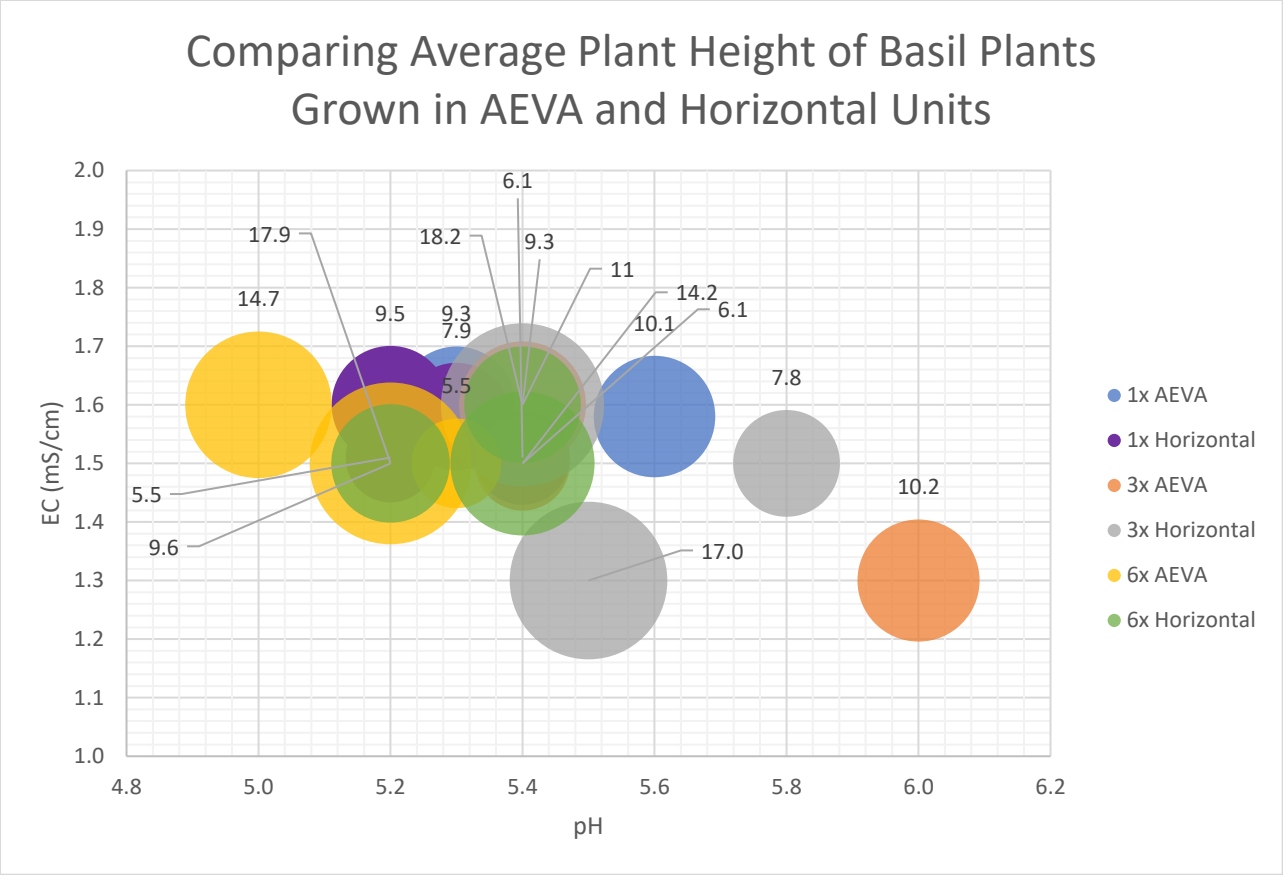


Figure 39.0: Comparison of the average plant height of basil plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Basil samples grown on the horizontal unit with a watering cycle of 3 times/day at a lighting regime of 18/6 shown the best overall growth.

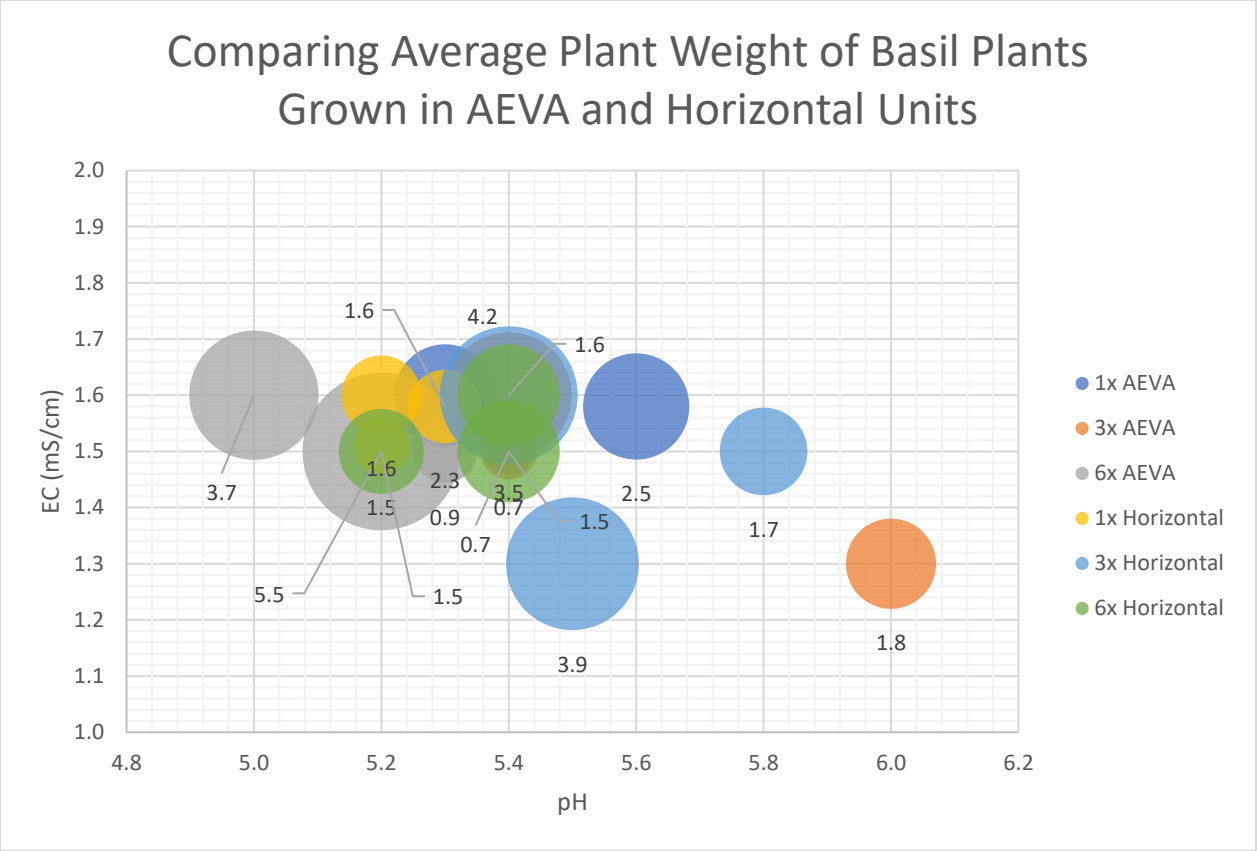


Figure 40.0: Comparison of the average plant weight of basil plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Basil samples grown on the AEVA with a watering cycle of 6 times/day at a lighting regime of 18/6 shown the best overall growth.

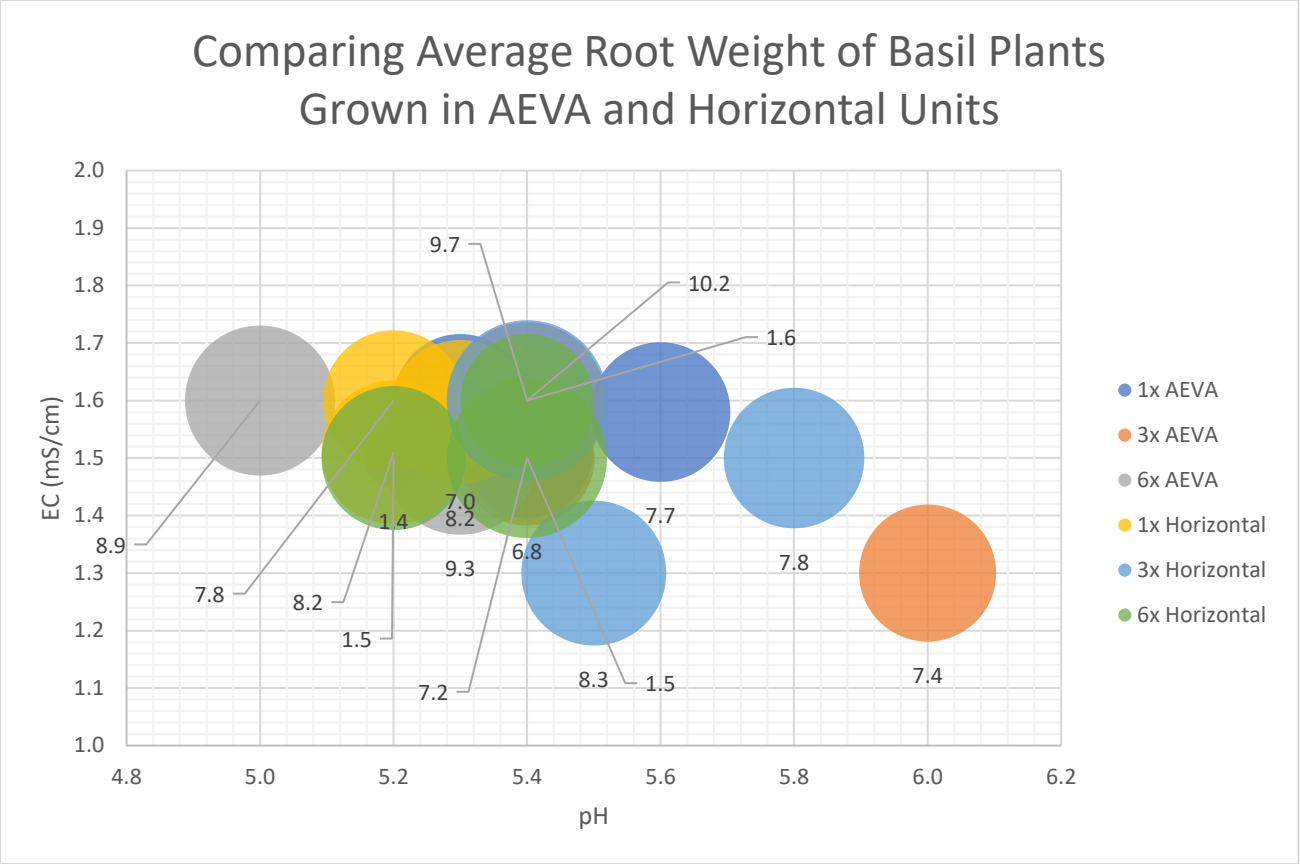


Figure 41.0: Comparison of the average root weight of basil plants grown in the AEVA and horizontal units to understand the impact of the EC and pH on the overall growth. Basil samples grown on the horizontal unit with a watering cycle of 3 times/day at a lighting regime of 18/6 shown the best overall growth.