



Plant performance in controlled environment agriculture, the effect of different ebb and flow irrigation intervals on *Brassica oleracea* and *Lactuca sativa*.

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Keywords: *Brassica*, Controlled-environmental Agriculture, ebb and flow, hydroponics, irrigation interval, kale, *Lactuca*, lettuce, plant factory, vertical farming.

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Abstract

Increasing productivity in controlled environment agriculture may decrease environmental footprint and increase profitability of food produced in a vertical farm. Irrigation may be a limiting factor when it comes to plant performance and relevant research is limited. This study aims to increase productivity of lettuce and kale in a vertical farm by finding what irrigation levels enable highest crop productivity. Crops were grown in stone wool substrate in an ebb and flow irrigation system under four different intervals (4, 15, 60, and 270 min). Results in fresh and dry weight indicate an optimal irrigation interval for lettuce between 5- and 15-min interval. This data is supported by mineral content of lettuce. No significant difference between these two smallest irrigation intervals can be found. For kale data is inconclusive and no optimal irrigation levels have been found. During the experiment airflow was hardest to control which may affected results.

Keywords: Brassica, Controlled-environmental Agriculture, ebb and flow, hydroponics, irrigation interval, kale, Lactuca, lettuce, plant factory, vertical farming.

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Abbreviations

| | | |
|------|---|--|
| A | = | photosynthetic rate |
| CEA | = | controlled-environment agriculture |
| GH | = | Greenhouse |
| GS | = | stomatal conductance |
| LED | = | light emitting diodes |
| L1 | = | layer 1 |
| L2 | = | layer 2 |
| L3 | = | layer 3 |
| L4 | = | layer 4 |
| NFT | = | nutrient film technique |
| PPFD | = | photosynthetic photon flux density |
| Ppm | = | parts per million |
| rH | = | relative Humidity |
| SLU | = | Swedish University of Agricultural Science |
| T | = | Temperature |
| VF | = | vertical farming |
| VPD | = | vapor pressure deficit |

1. Introduction

Urbanization may lead to concentrated areas of high demand, creating competition for housing, work locations, transporting and green spaces. It may also lead to an increasing demand for fresh products like, salads, herbs, and other leafy greens in for example urbanized areas. Due to the expansion of cities through urbanization, high productive land, which is 1,77 times more productive compared to average soil, is no longer used as arable land. Main reason being that most productive agricultural land is placed near the urban areas (Bren d'Amour et al., 2017). A study suggests that in 2050 arable land per person is 33% of that in 1970 (Nations, 2017). One solution to this problem could be to increase our knowledge on field or greenhouse production, located outside cities, and focusing on post-harvest treatment to ensure its supply through transportation. But because of urbanization the interest in urban agriculture has also increased (Nations, 2014).

Logically, food production could be located closer to its demand. Due to the high demand on available land within cities a solution could be found in controlled environment agriculture (CEA). In Sweden, vertical farming (VF) has the potential to be twice as efficient as the most high-tech greenhouse installation to date (Graamans et al., 2018). Currently in Sweden, a plant factory would use 1411 MJ/kg dry weight compared to greenhouse production with artificial illumination needing 1699 MJ/kg dry weight (Graamans et al., 2018). VF, being placed under the umbrella of CEA, can be described as a place where crops grow closely together most often vertically positioned within in a purpose-controlled environment. Other words to describe VF or CEA are, indoor farming, and or plant factories. A study from 2022 suggest that VF could play a key role in the transition towards a more sustainable society by reduced CO₂ emissions due to decrease transportation and a limited post-harvest supply chain (Vatistas et al., 2022).

However, VF is not yet widely accepted. According to two environmental impact assessments products from vertical farms currently have a higher environmental footprint compared to greenhouse farming and soil production. Doing such life cycle analysis allows for the highlighting if impact potentials. Both studies conclude the highest contributor is its electricity usage per plant produced (Martin and Molin, 2019, Blom et al., 2022). Both scenarios taken into considerations are case studies within a developing industry. Reducing the environmental impact may be achieved by integration of VF within existing or future infrastructure. (Martin et al., 2022). Biggest conditubers being electricity usage and building costs due to proximity and host-building synergies (Shao et al., 2021)

Profitability of VF may not yet be on the same level as greenhouse or field production, the main consensus has always been that VF cannot fight economically due to high costs of powering artificial light (Shackford, 2014). However, hypothetically a vertical farm compared to a greenhouse in Quebec, Canada, may have a very similar profit model, due to high dependency of greenhouses on land availability and its price to be profitable. (Eaves and Eaves, 2018). Due to the advancement of light emitting diodes (LED) productivity may increase more in VF than in greenhouses. (Eaves and Eaves, 2018). Farming as a service, a business model that allows farmers to produce a service on a pay per unit or subscription basis, is suggested as a solution to profitability issues. (Martin and Bustamante, 2021). One business that already has developed this approach and part of this research is SweGreen AB.

Technological improvements have always been the driver within VF. Within most systems, available on the market today, environmental variables affecting a plants behaviour can be controlled. These abiotic factors controlled inside a VF are, light (both spectrum and quantity), relative humidity (rH), temperature (T), airflow, and carbon dioxide concentrations (CO₂). One way to tackle profitability and electricity usage issue is to increase crop productivity, selling more plants for less electricity (Moghimi and Asiabanpour, 2023). This can be achieved by implementing or developing technologies enhancing crop growth and reduce energy consumption (van Delden et al., 2021). Most vertical farms also have highly controlled nutrient management system including different types of irrigation. Control of these factors allow for a more predictable plant composition and growth rate (SharathKumar et al., 2020). The development of light, especially within the technological development of LED's is a well-covered research topic. An example is a comparison of light use efficiency between a vertical farm, greenhouse, and field production, for the cultivation of lettuce. Conclusion was that VF produced the most biomass per emitted light over time, 0.55 g/mol compared to 0.39 g/mol for a greenhouse. Figures compared to field production almost doubled (Jin et al., 2023). Since VFs enable to possibility of controlling most available plant conditions research complexity may increase too. A study mapped out plant responds (lettuce) to CO₂ concentration, light quantity, and air speed. Increased light intensity enhances plant growth under high CO₂ conditions but at lower CO₂ conditions it has no effect or inhibits growth (Ahmed et al., 2022). It is important to not focus on one conclusive result on what is best given these settings, but produce research that enables another research to increase this limit. Optimized growing conditions in combination with predictability and consistency are main drivers of these innovations.

Since water use efficiency of hydroponics compared to soil production is efficient, it might not have been of great interest yet. Tomatoes grown in two hydroponic set ups transpired less water and had a higher water efficiency compared to plants grown in soil (Verdolina et al., 2021). Furthermore vertical farming technology may increase water use efficiency even more partly due to the technological advancement of vertical farms enabling the recycling of transpired air through condensation (Pacak et al., 2020). Such system is adopted by the commercial CEA farm SweGreen in its operation, claiming water use efficiency up to 95%. It is important to

study irrigation management in CEA since this might become the limiting factor in optimizing plant performance.

To monitor plant response, this research focusses on lettuce, *Lactuca sativa*, and kale, *Brassicae oleracea*. Lettuce because it is a popular research crop within the field of vertical farming. Of all crops related research 29% included lettuce, making it the most predominant within vertical farming (Najera et al., 2023). Kale is chosen because of the importance of finding and exploring alternative sources of vitamins, fibres, and minerals that can be produced hyper locally within CEA.

1.1 Project Objectives and Aim

For the vertical farming to be competitive, more research will be required. To ensure vertical farming/controlled-environmental agriculture in the future it is suggested to bring closer together research and collaborations that focus on product optimisation. (Oh and Lu, 2023) One example could be by a collaboration between industry and academics. This paper aims to bring CEA research closer together by publishing all information applicable. Data monitoring of as many variables as possible must be shared, additionally increasing trustworthiness of this research., must be shared to enhance this and future collaborations (Oh and Lu, 2023). Most universities like, SLU, do not have (yet) a vertical farm that enables a test like this. The industry holds most knowledge on constructing this specialized machinery. Since lots of research in vertical farming will be conducted by industry the importance of sharing all available monitored data should be emphasised. For SweGreen it is important find out with parameters of the controlled environment are least trustworthy.

Thus, from a sustainable perspective irrigation management might not yet have been the most imminent desired research topic. However lacking research on irrigation management may slow the entire progress on high plant performance in a controlled environment. Aim of this research is to identify, considering a plant physiological perspective, plant's reaction under different ebb and flow irrigation intervals under high performance growing conditions.

1.2 Research question

Questions that this research attempts to answer:

- What irrigation interval enables highest crop productivity for lettuce and kale that are grown in controlled environment agriculture?
- What effect do different irrigation intervals have on the mineral content of lettuce and kale that are grown in controlled environment agriculture?

The collaboration between Swedish university of Agriculture (SLU) and SweGreen AB enables testing under advanced growing conditions. Research is enabled by growing lettuce and kale under different irrigation conditions and combining this with SLU available resources. The research facility of SweGreen, SweGreenX, located in Stockholm is used for the duration of the experiment.

1.3 Hypothesis

Based on the literature available a few assumptions are expected to be found.

- It is expected to find significant differences for both Lettuce and Kale because of different ebb and flow irrigation intervals
- Lettuce is expected to thrive better under more frequent irrigation intervals
- A Kale plant is expected to thrive under less frequent irrigation intervals

2. Method & Materials

2.1 Experimental design

The experiment was conducted at the research and development area of SweGreen AB, SweGreen X, in Stockholm. A four-layer vertical farm built by SweGreen and placed inside a climate-controlled room was used for all growing stages of the experiment. Volume of the climate control room was 48m³. Each growing layer had the following dimensions, 630 mm x 2130 mm. To control temperature and humidity inside the growing room, a split air conditioner SC-JA4819, manufactured by Qlima in the Netherlands, was used. To ensure adequate air circulation on all the layers of the vertical farm four 15watt electrical ventilators were placed.

Plants used in this experiment were *Brassica oleracea L.*, green kale (cultivar Winnetou F1) , and *Lactuca Sativa L.*, green oakleaf (cultivar Freelou). All plant were grown on a stone wool substrate, size 36/36/40mm. The nutrient solution used in this experiment was an in-house mix of dissolved mineral salts, containing a N:P:K:Ca ratio of 7:1:9:5. Electroconductivity (EC) of the nutrient solution was balanced around 2.1 mS/cm and pH levels balances around 5.8. To control the EC, flow rate and temperature of the water, picomag electromagnetic flowmeter from Endress + Hausser (*Switzerland*) was used. For pH control, (acidifying the solution using phosphoric acid) Digiline ORP from JUMO, Germany, was used.

Each layer in the VF represented a different irrigation interval, a total of four different treatments were tested. (Table 1) Irrigation was conducted by the principle of ebb and flow technique; a layer would fill up to a height of 14 mm before being drained.

Table 1: Irrigation interval for each layer in the experimental set up.

| | Irrigation interval under light conditions (min) | Irrigation interval under dark conditions (min) |
|--------------|--|---|
| Layer 1 (L4) | 4 | 180 |
| Layer 2 (L2) | 15 | 180 |
| Layer 3 (L3) | 60 | 180 |
| Layer 4 (L4) | 270 | 180 |

The plant in this experiment were grown under a LED lighting solution, Siera from Heliospectra, Sweden. Photoperiod in this experiment was 18 hours. Light intensity was set at 350 ppm 60 cm from light source. Before the start of the experiment the lights on all the four layers were individually analysed using, PAR200 Quantum Spectrometer manufactured by UPRtek, Taiwan. The spectrometer was placed in the middle of each layer and set to analyse for, photon flux density (PFD) in $\mu\text{mol}/\text{m}^2/\text{s}$. For the collection of raw data PAR200 Plus version 1.0.0 mobile phone application from UPRtek, Taiwan, was used.

Two sets of experiments were conducted within this experimental set up. Both experiments used plant seedlings raised in an external nursery provided by SweGreen. Kale was seeded manually using 1 seed per plug and placed in germination room for 2 days. Lettuce was seeded manually and directly placed in the nursery. Both seedling types were 21 days old when used the experiment started. Both experiments had the same plant density, 77 plants per growing layer, 38 kale and 39 lettuce seedlings. Experiments were repeated due to improvements found during experiment 1.

Experiment one (E1) corresponds to a total growing period of 15 days for both crops. Starting date of E1 was 23 January 2023. A total of 29 kale samples were taken from each layer during E1. A total of 27 lettuce plants were taken during E1 from each layer. Plants growing on the edge of the growing area were discarded when harvested.

Experiment two (E2) corresponds to a total growing period of 17 days for kale and 21 days for lettuce. Starting date of E2 was 21 February 2023. A total of 24 plants from both species were taken during E2 from each layer. Plant growing on the edge of the growing area, this time including kale and lettuce closest to each other, were discarded when harvested.

2.2 Measurement during the experiment

At the beginning of the experiment settings for controlled abiotic factors were established. Of all parameters considered in CEA the temperature, relative humidity, light quantity and spectrum, and air velocity were set to industry standard. All parameters presented in table 2, including light and carbon dioxide (CO_2) levels, were monitored on a weekly base. Humidity and temperature were monitored collected by thermohygrometer 605i (Testo, Germany). Air velocity was set and monitored using a hot-wire anemometer 405i (Testo, Germany). To analyse measurements on humidity, temperature and air velocity Testo smart mobile (application version 17.7.11.70136). To monitor levels of CO_2 a TFA Air CO_2 ntrol mini (Dostmann, Germany. For both experiment, temperature and humidity data logger 176h1 (Testo, Germany), was used to collect data on temperature and humidity difference during light on and off period. Testo comfort Software (basic 5.6 SP6.3.167.36094) was used for retrieving data from this logger. All data was collected around 11 am every week.

Table 2: set parameters at the beginning of the experiment.

| Parameters | Set values |
|-----------------------|------------|
| Temperature (C°) | 23.5 |
| Relative humidity (%) | 60 |
| Air velocity (m/s) | 0.5 |

An analog moisture meter was used in this experiment. This meter, specialized for organic soils, was tried on a rockwool growing medium. For all kale treatments 20 samples were taken, 10 samples two minutes before and 10 samples after planned irrigation.

2.3 Gas exchange analyser

To analyse the photosynthetic rate (A) ($\mu\text{mol}/\text{m}^2/\text{s}$) and stomatal conductance of CO_2 (GS) ($\text{mol}/\text{m}^2/\text{s}$), a Portable Photosynthesis system was used (LCPro, ADC bioscientific, Hoddesdon, UK). Ambient levels for CO_2 , temperature and humidity were used. The LED-light modular extension was used and photosynthetic photon flux density (PPFD) set on $350 \mu\text{mol}/\text{m}^2/\text{s}$. Data was manually read from the device after being placed on the plant's biggest leaves for 15 min. For each layer and both species technical replicates of three were made. On day 13 of E1, samples from kale leaves were taken. On day 14 of E1, samples from the lettuce leaves were taken.

2.4 Post-harvest analyses

After each treatment plants were harvested. Roots and rockwool growing medium were removed by using a scissor to cut off the main stem that levelled with the top of the rockwool plug. Samples from E2 were individually tagged and noted for its fresh weight and dry weight using an electronic scale (sensitivity of 0,5g). Each individual sample for each layer were dried at $65 \text{ }^\circ\text{C}$. Kale was dried for a period of 48h. Lettuce samples were dried for 96h. From E2 lettuce replicates of three individual samples were taken for each layer and sent to the lab for analysis. For E2 kale replicates of three for layers 1 and 4 were taken and sent to the lab for analysis.

2.5 Plant analysis

Dried samples were analysed by a commercial lab (LMI AB, Helsingborg, Sweden). Quantitative determination of nitrogen was determined by the Dumas method. To determine dry matter percentage samples were tested according to Swedish Standard (SS) 28113.1 The samples were analysed with respect to; aluminium, boron, calcium, copper, iron, potassium,

¹ <https://www.sis.se/en/produkter/environment-health-protection-safety/water-quality/sewage-water/ss28113/>

magnesium, manganese, molybdenum, sodium, phosphorus, sulphur, silicon, and zinc. This using a ICP OES spectrometer according to SS 28311.2

2.6 Data analysis

Weekly collected data on temperature, air velocity, humidity, and carbon dioxide was further analysed by taking the percentage its biggest difference to average. This to monitor the controlled parameters inside the vertical farm.

All data was processed through Microsoft Excel for Mac version 16.72. Representation of tables and figures generated through the same program. Statistical analysis was made using Minitab software (version 21.0.0). Within this software, standard deviation, analysis of variance (ANOVA), and Tukey's multiple comparison test with a 95% confidence rate, were used.

² <https://www.sis.se/api/document/preview/8025632/>

3. Results

3.1 Light

The effective photon flux density for wavelength between 380 and 780 are given. (Figure 1) Data represented is an average from the four growing layers. Results showed an effective light ratio of 1,4 for blue to green, 6,22 for red to far-red, and 4,06 for red to blue. Data presented in this figure is presented as average based on measurement from all the four layers in the vertical farm. Raw data shows that error margin of each individual layer was within 5% of the average.

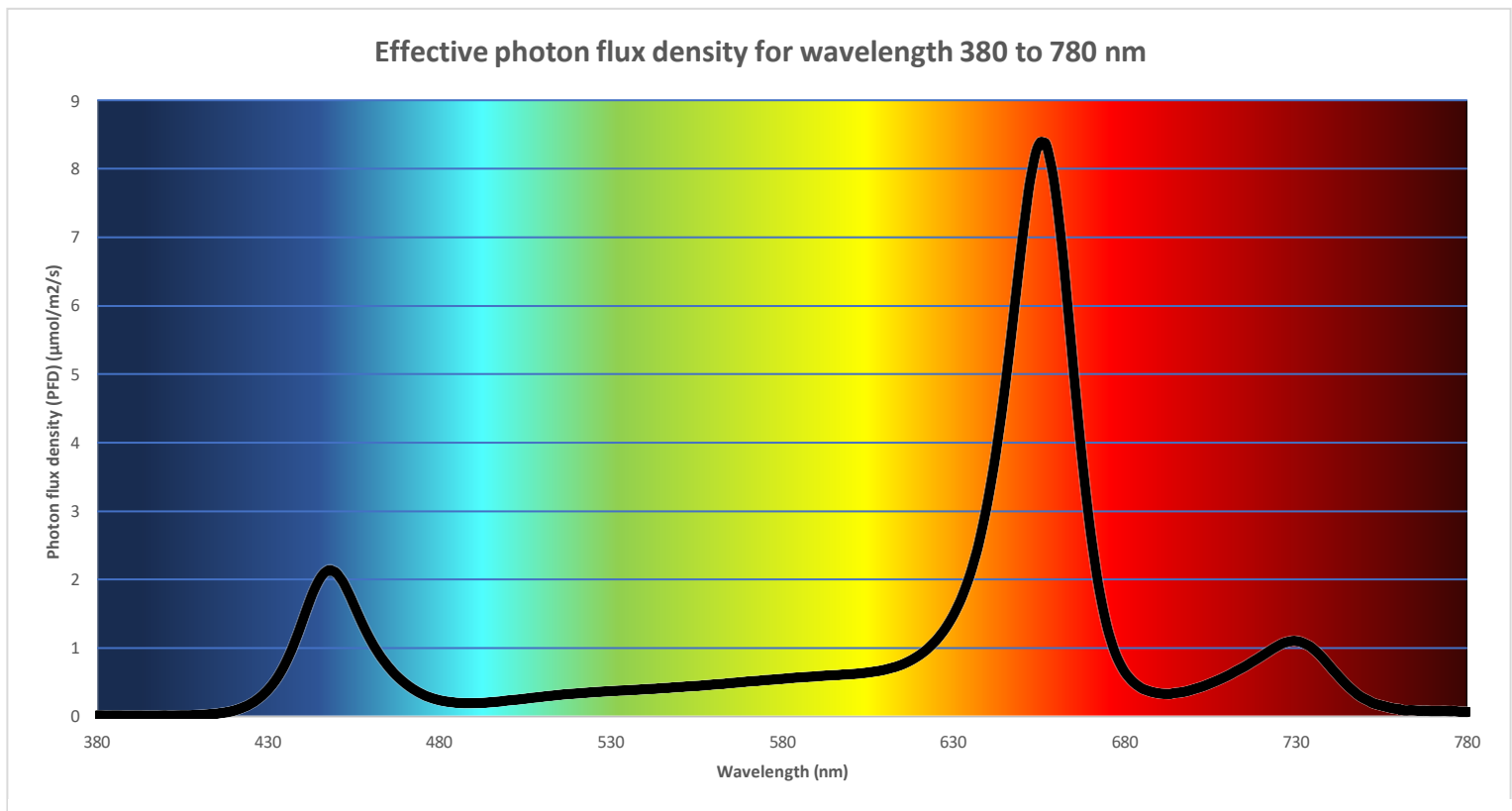


Figure 1: Representation of the four test bed layers showing the effective photon flux density for wavelength between 380 and 780 nm with data gathered using a quantum spectrometer

3.2 Climate control

3.2.1 Overnight monitoring

Data on relative humidity and temperature recorded during week 1 and 5 of the experiment were presented. (Figure 2 and 3) Data was gathered to monitor the different conditions during light on and off period. Light off period was between 0:00 am and 6.00 am. During this period in both the graphs temperature decreases around 3,5°C. Accordingly, the relative humidity increases around 10-12% with lights are off.

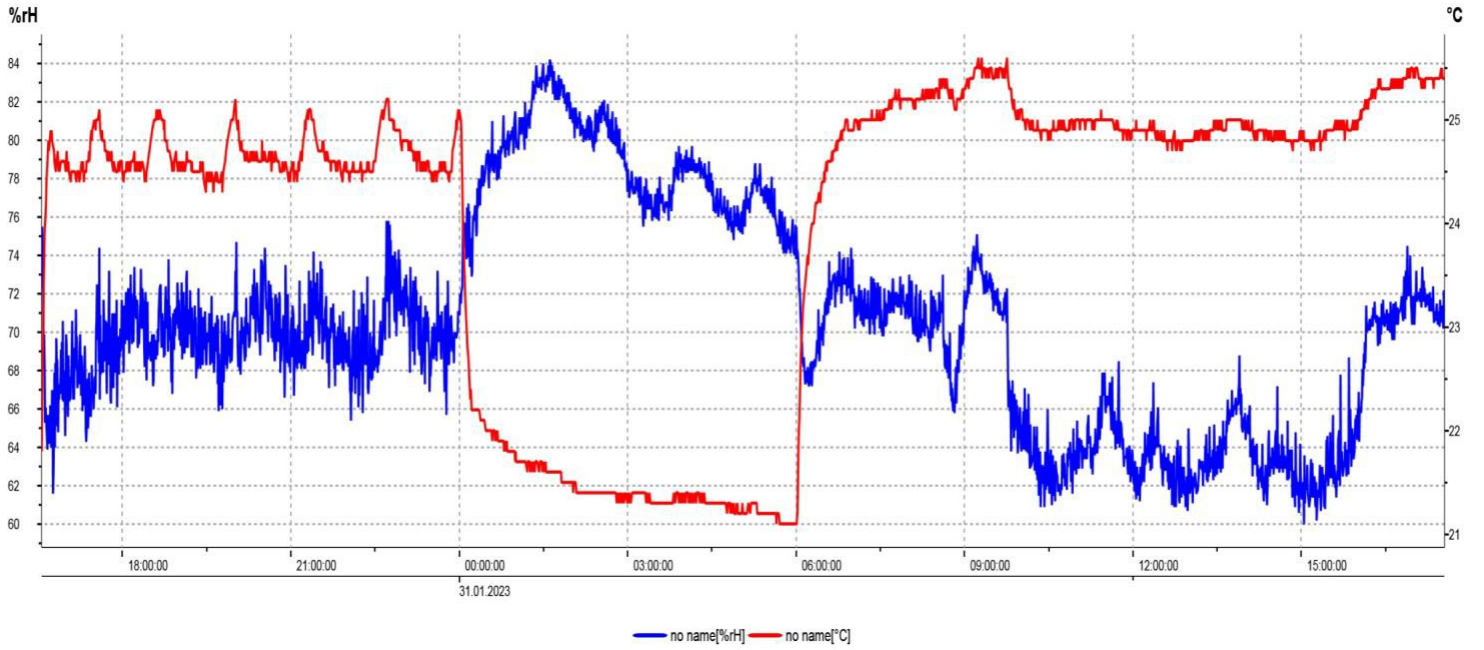


Figure 2: Adaption from graph created by testo comfort software basic 5.6. Collection of 18 hours of data on temperature and humidity retrieved from a testo 176h1 data logger in a vertical farm set up. Data collected for the beginning of experiment 1

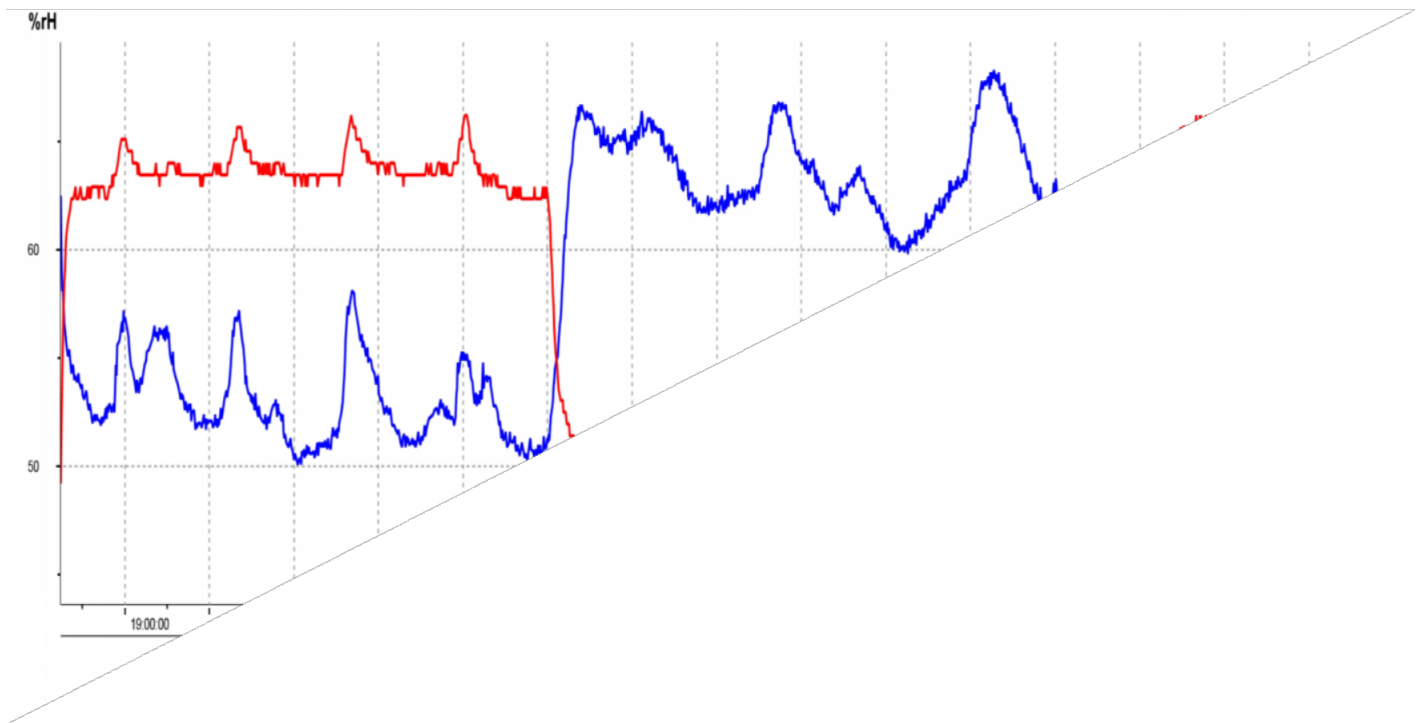


Figure 3: Adaption from graph created by testo comfort software basic 5.6. Collection of 18 hours of data on temperature and humidity retrieved from a testo 176h1 data logger in a vertical farm set up. Data collected for the beginning of experiment 1

3.2.2 Weekly monitoring

Climate data for the entire duration of the experiment is presented. On a weekly base data on airflow (figure 4), temperature (figure 5), relative humidity (figure 6), and carbon dioxide levels (figure 6).

Highest levels of airflow (0,65 m/s) were monitored on layer 4 during week 2. Lowest levels of airflow, 0,2 m/s, were monitored on layer 5 during week 5. Average windspeed during the experiment was between 0,39 – 0,56 m/s. Range of the average windspeed for the entire duration was between 0,39 and 0,56 m/s. The biggest difference in airflow between layers, 0,35 m/s, within the same week was during the initial set up of the experiment.

Highest temperature (24,8°C.) was recorded during week 5 on layer 1. Lowest temperature, 22°C., was recorded week 2 layer 4. In all weeks, except week 4 temperature decreases with increased height of the layers. 1 being the lowest positioned layer and 4 being on the highest. During week 1 temperatures were stable across most layers. Average temperature for the entire duration of the experiment was between 22,7°C and 24°C. Biggest temperature difference between layers, 1,6 C, within the same week was recorded week 2.

Highest relative humidity, 71.1%, was recorded on layer 4 during week 6. Lowest relative humidity, 50%, was recorded layer 4 week 0 and week 4 layer 1. Overall trend indicates increased relative humidity with increased number of weeks. Average relative humidity for the entire duration for the experiment was between 51.5 % and 68.8%. Within the same week biggest difference, 6.4%, was recorded week 6.

Highest amount of CO₂ as part per million (ppm), 702, was recorded week 6 on layer 4. Lowest amount of CO₂ as part per million, 559, was recorded week 4 on layer 1. Biggest difference in CO₂, 80ppm, was recorded week 2 between layer 1 & 2. Average concentration of CO₂ during the experiment were between 556 – 685 ppm.

Average data of the weekly measurement parameters for the entire duration of the experiment, biggest difference, and relationship between those two were calculated and presented (Table 3). Statistically airflow fluctuated the most compared to its average, and temperature the least.

Table 3: Average levels, biggest difference, and relationship between difference and average for airflow, temperature, relative humidity, and carbon dioxide concertation for the entire duration of the experiments.

| | Average | Largest difference | Relationship difference to average (%) |
|-------------------------------------|---------|--------------------|--|
| Airflow (m/s) | 0,46 | 0,35 | 76,1 |
| Temperature (°C) | 23,8 | 1,6 | 6,7 |
| Relative humidity (%) | 59,4 | 6,4 | 10,8 |
| CO ₂ concentration (ppm) | 636 | 80 | 12,6 |

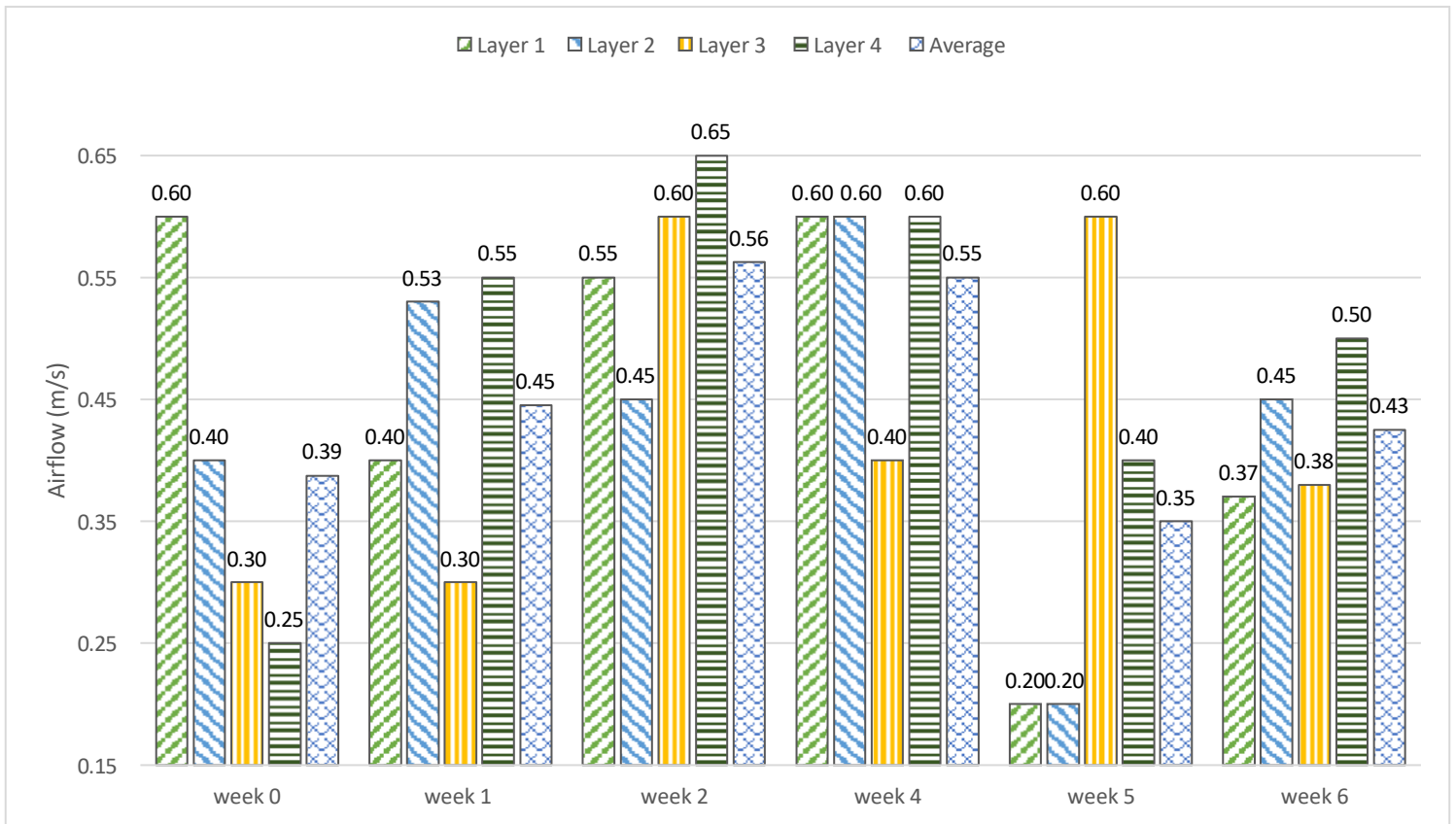


Figure 4: Difference in airflow per growing layer per week of the experiment. Week 0 represent the initial conditions of the experiment. During week 1 & 2 experiment 1 was conducted. Week 4-6 represents experiment 2.

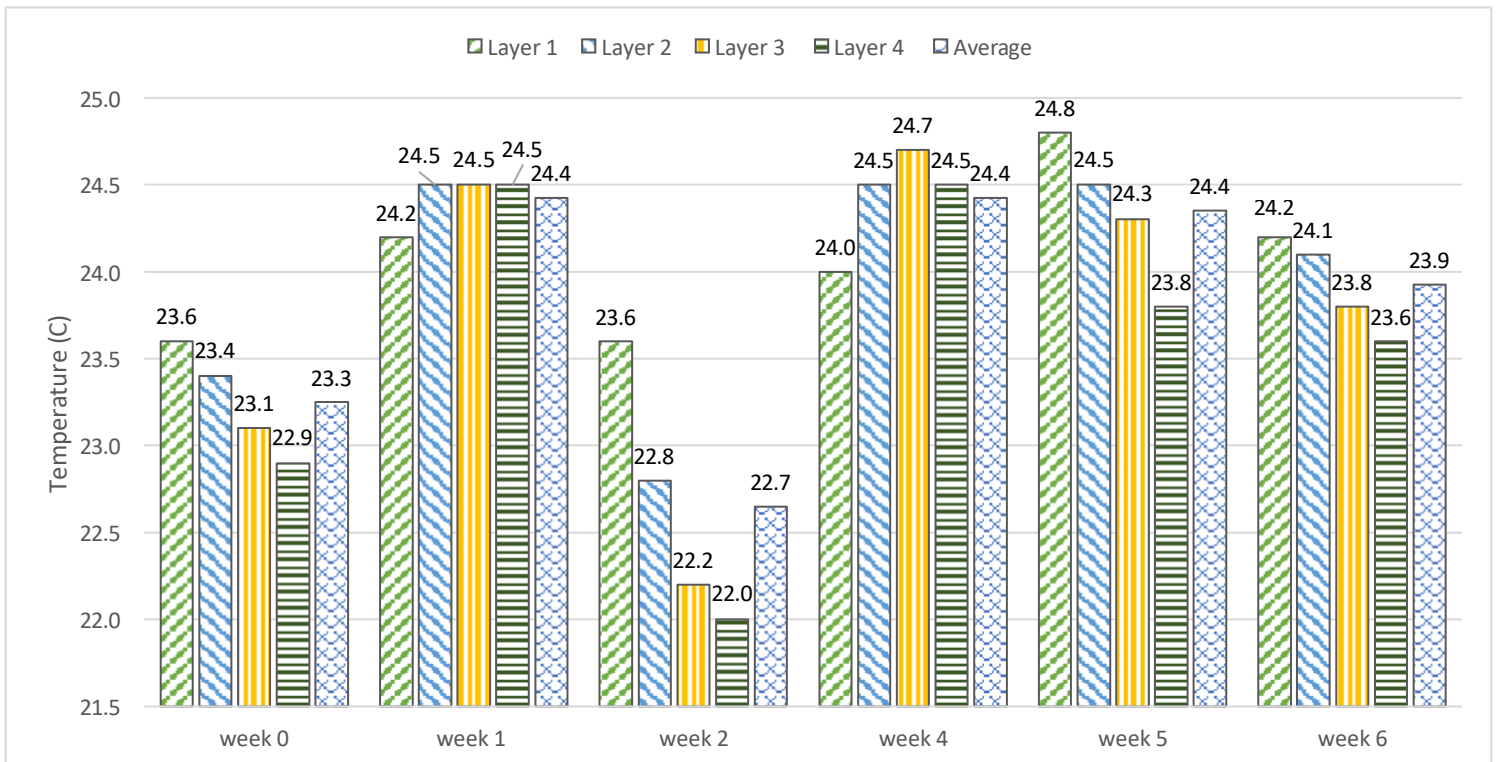


Figure 5: Difference in temperature per growing layer per week of the experiment. Week 0 represent the initial conditions of the experiment. During week 1 & 2 experiment 1 was conducted. Week 4-6 represents experiment 2

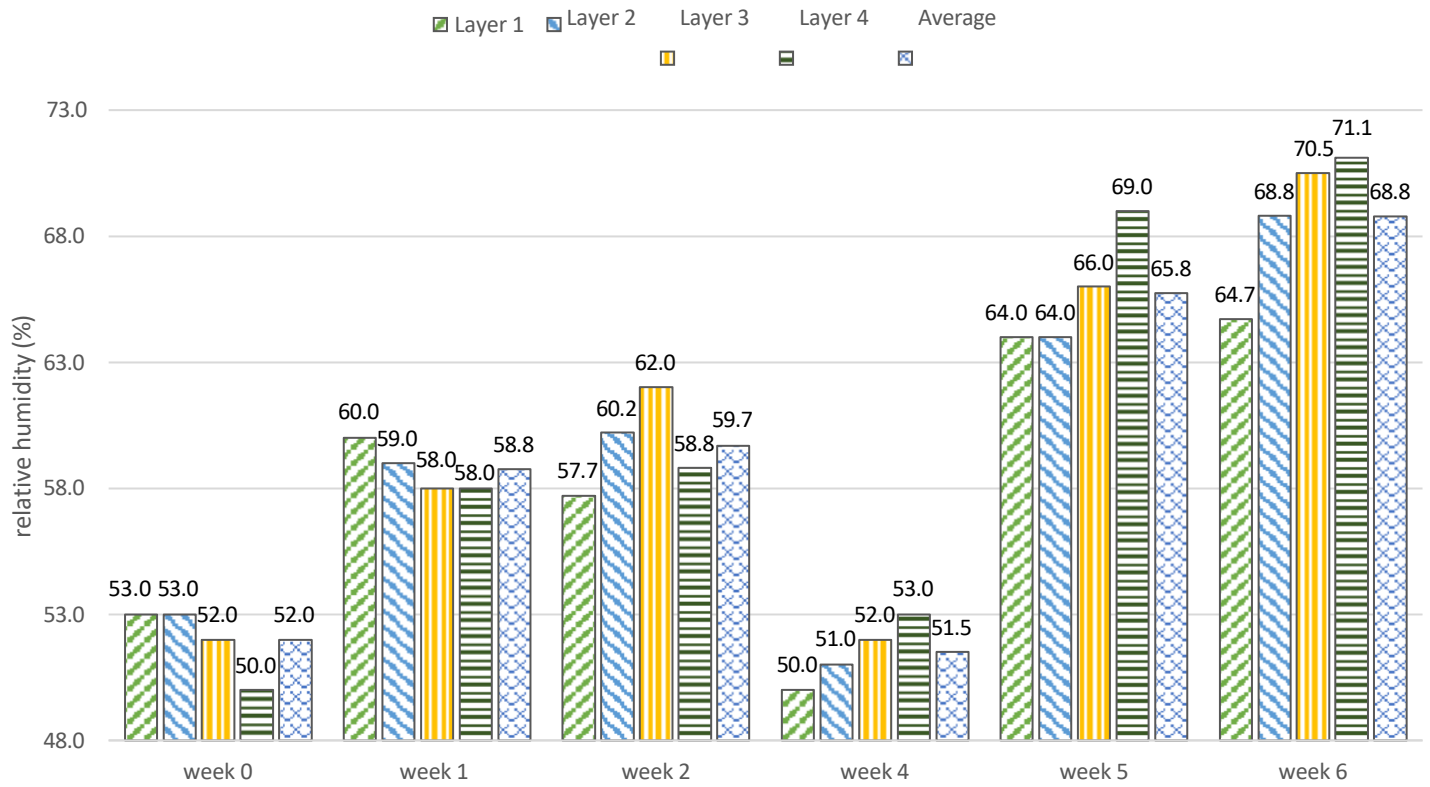


Figure 6: Difference relative humidity per growing layer per week of the experiment. Week 0 represent the initial conditions of the experiment. During week 1 & 2 experiment 1 was conducted. Week 4-6 represents experiment 2

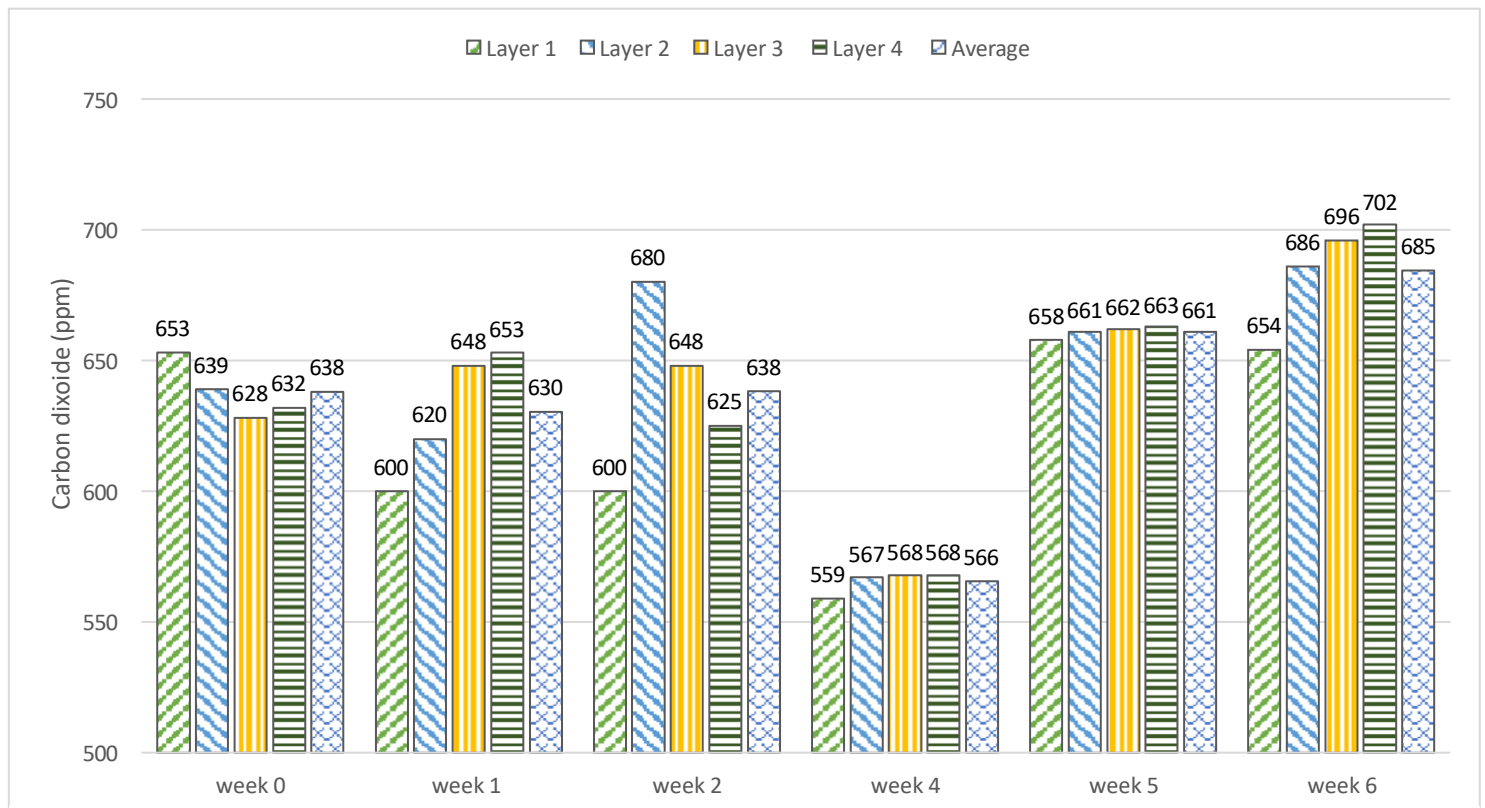


Figure 7: Difference CO₂ concentration per growing layer per week of the experiment. Week 0 represent the initial conditions of the experiment. During week 1 & 2 experiment 1 was conducted. Week 4-6 represents experiment 2

3.3 Moisture within growing medium

Different voltage potential of rockwool medium 2 min before and 2 min after for four different irrigation intervals are presented. Standard deviation for each treatment were included. (Figure 8) For all irrigation intervals moisture content increases 2 min after irrigation, biggest difference between layers 3 and 4. Overall highest moisture levels corresponds to shortest irrigation interval represented in layer 1 and 2. Highest voltage potential of 778,2 was measured 2 min after 15 min irrigation interval. Layers 3 and 4 show lower moisture capacities both before and after irrigation compared to layer 1 and 2. Lowest overall voltage potential was measured 2 min before 60 min irrigation interval. Standard deviation is highest for irrigation 2 minutes prior for layers 3 and 4. Overall standard deviation is higher at plugs on layer 3 and 4.

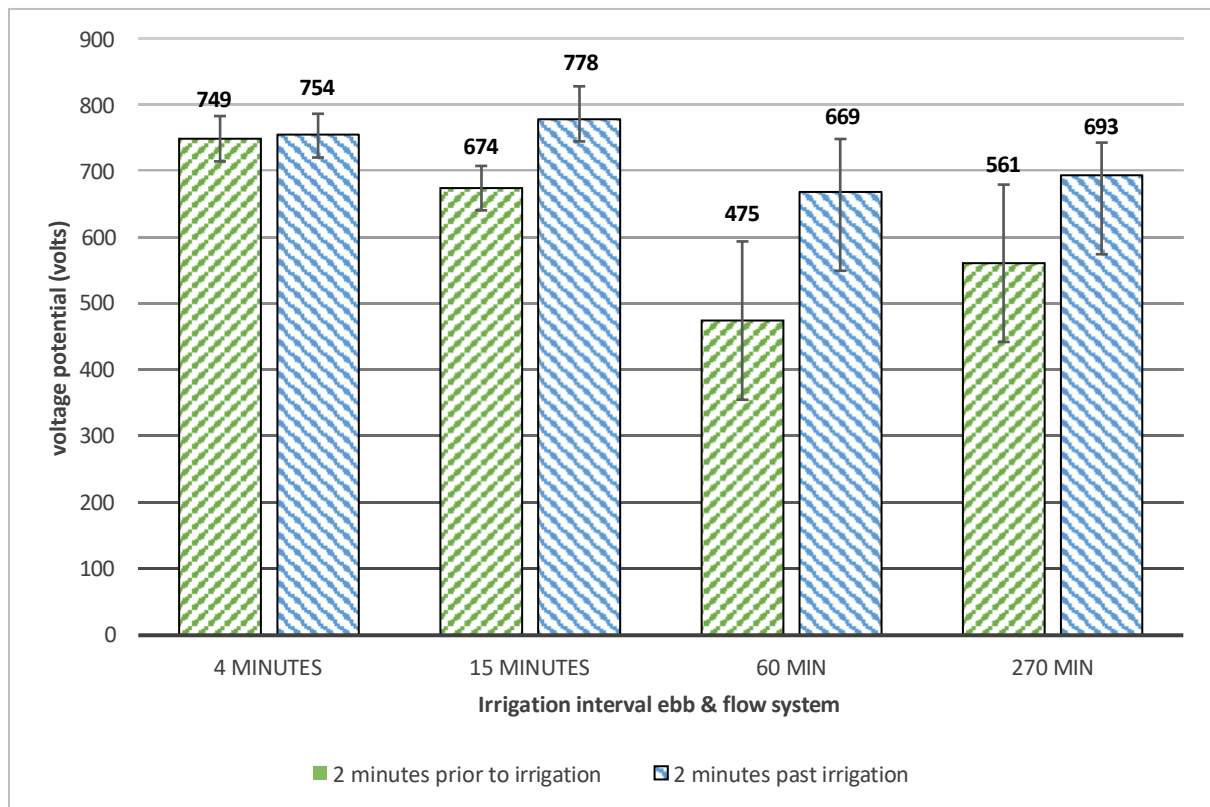


Figure 8: Voltage potential of rockwool growing medium seeded with kale under four different irrigation intervals for 2 minutes prior and 2 minutes after irrigation within an ebb and flow system. Error bars represent standard deviation

3.4 Photosynthetic rate & stomatal conductance

The photosynthetic rate (A), expressed in $\mu\text{mol CO}_2/\text{m}^2/\text{s}$, and stomatal conductance (GS), expressed in $\text{mmol}/\text{m}^2/\text{s}$ were collected. (Figure 9) Both lettuce and kale are represented and for each data set standard deviation was given. For Kale, A is higher compared to lettuce in all irrigation intervals. Highest overall A is for Kale under 4, 15, 60 min interval. Highest overall A for lettuce was found during the 60 min interval. For both species lowest A was found under 270 min interval.

GS was found to be highest for both Kale and Lettuce depending on irrigation interval. Overall highest GS was found at 4 min interval for lettuce. Lettuce GS was the lowest at 270 min interval. GS for Kale found to be the highest at 60 min and lowest 4- and 270- min interval.

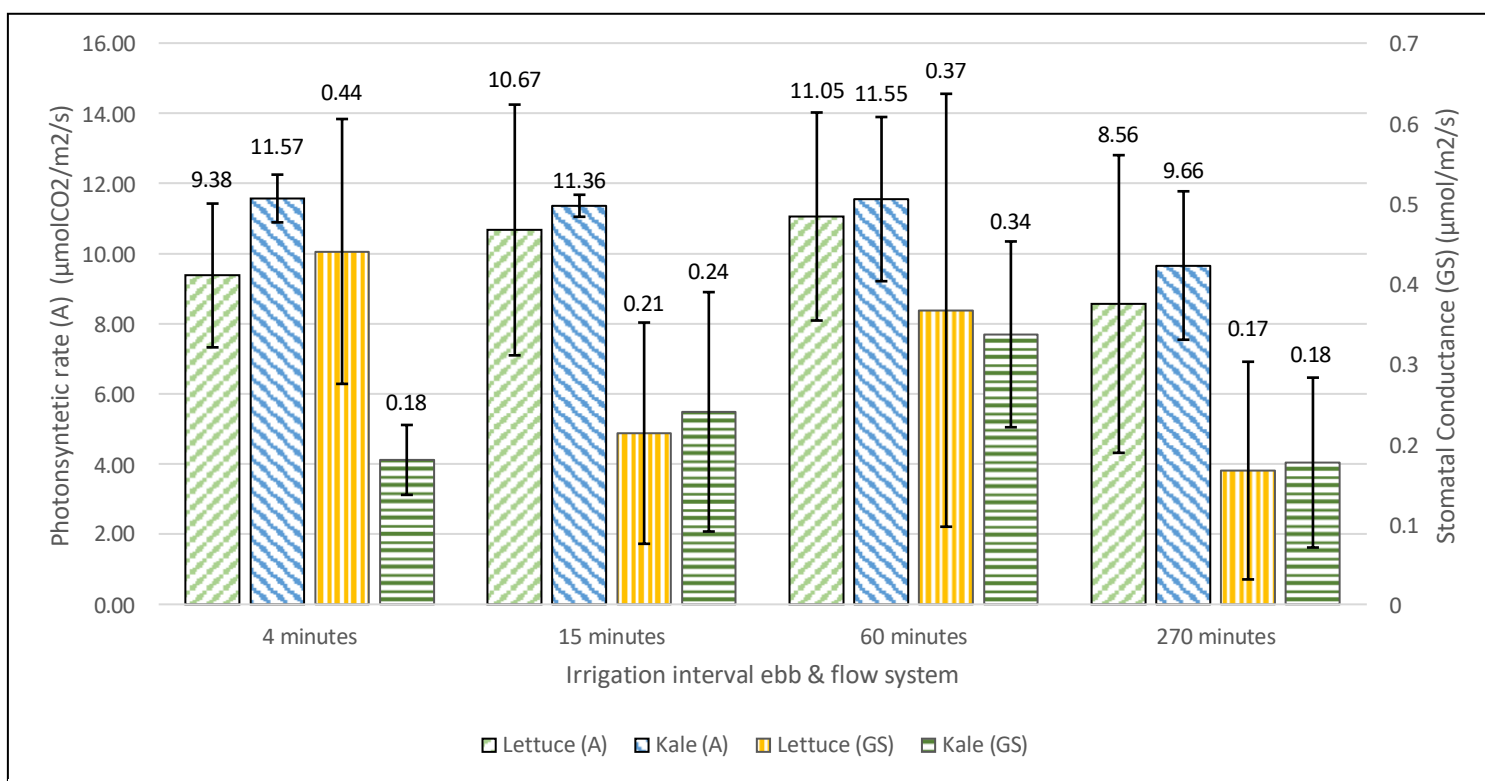


Figure 9: Photosynthetic rate (A) expressed as $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ and stomatal conductance (GS) expressed as $\text{mmol}/\text{m}^2/\text{s}$ for lettuce and kale under four different irrigation intervals. Error bar represents standard deviation.

3.5 Fresh & dry weight

Fresh and dry weight of lettuce and kale under four different ebb & flow irrigation levels were presented, standard deviation of each data set is represented as error bar, probability is given for each parameter, and significant difference represented as different letters. (Figure 10) Fresh weight of lettuce, dry weight of kale, and dry weight of lettuce showed a relative low value for indicators of probability namely, 0, 0, and 0,001. Fresh weight of kale had a high value for indicator of probability, 0.168. No significant difference between irrigation intervals for fresh weight of kale was found. A significant difference for fresh weight of lettuce was found between irrigation interval of 270 min, 60 min, and the latter two. The latter two, 4, and 15 min did not show a significant difference to each other. Two significant differences for dry weight of kale were found. First difference between 60 min irrigation interval and 4 + 270 min. Another difference was found between 15 min and 270 min irrigation interval. A significant difference for the dry weight of lettuce was found for irrigation intervals of 270 min compared to 4 and 15 min. No other significant difference for the dry weight of lettuce were found.

Overall highest fresh weight lettuce was found under 4 min irrigation interval, 129,33 g. Lowest fresh weight was found under 270 min, 56,77 g. Overall dry weight for lettuce was found to be highest for 4 min irrigation interval, 5,52 g and lowest for 270 min interval 4,35 g. For kale highest dry weight were found under 4- & 270-min irrigation intervals, around 2.9 g. Lowest dry weight was found at 60 min irrigation interval, 1.94 g.

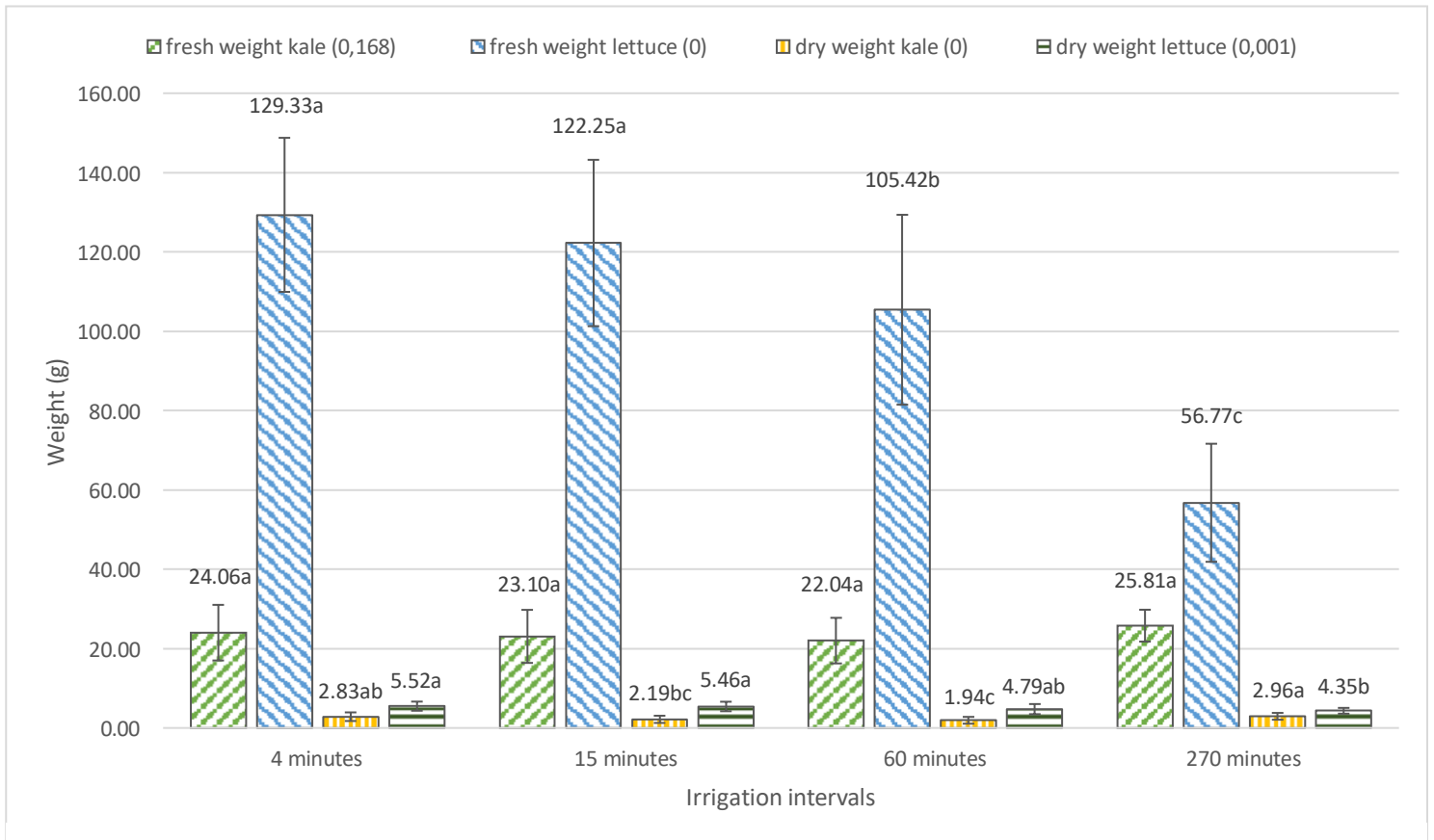


Figure 10: Fresh and dry weight (g) lettuce and kale for four different ebb and flow irrigation intervals. Error bars represent standard deviation and different letters above the columns and next to data label within different measurements indicate a significant difference ($\alpha = 0,05$) between different irrigation intervals. Value after each element given between brackets indicate the probability of obtaining the observed results. Expressed a p-value in ANOVA.

3.6 Mineral content

Aluminium, boron, calcium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, phosphorus, sulphur, silicon, and zinc were the minerals analysed for the purpose of this research. Traces of molybdenum were too small to be accurate results for both lettuce and kale. For all samples of kale traces of aluminium were too small to be accurate. Both elements are thus excluded from further representation of results. Mineral content always represented as milligram per kilogram dry weight but separated into two figures, representing absolute values 0-90 mg/kg (figure 11 and 12), and values 0-10000 mg/kg. (Figures 13 and 14)

No significant differences were found for aluminium, iron, sulphur, manganese, and silicon.

Significant differences for magnesium, sodium, copper, and zinc were found for kale under two different irrigation intervals, 4 min, and 270 min (figures 11 and 12). For all mineral that were significantly different absolute content was found to be higher under 270 min irrigation interval compared to 4 min. Other elements analysed, nitrogen, calcium, potassium,

phosphorus, and boron did not show a significant difference. Taking this into account all elements except nitrogen, calcium, and phosphorus showed absolute higher mineral content per kg of dry weight for the higher irrigation interval.

Significant differences for nitrogen and potassium were found for lettuce under four different irrigation intervals, 4 min, 15 min, 60 min, and 270 min. (Figures 13 and 14) Nitrogen mineral content was found to be highest under least frequent irrigation interval. Values for 4 min irrigation interval showed a significant difference compared to 270 min interval. Mineral content of potassium in lettuce is highest under irrigation intervals of 4 min and 15 min. This data is significant different to irrigation interval of 60 min and 270 min. Under 270 min potassium content is lowest. Other mineral content found (calcium, magnesium, sodium, phosphorus, boron, copper, and zinc) do not show a significant difference. However, data suggest a declining content of minerals with decreasing intervals of irrigation except for sodium and boron.

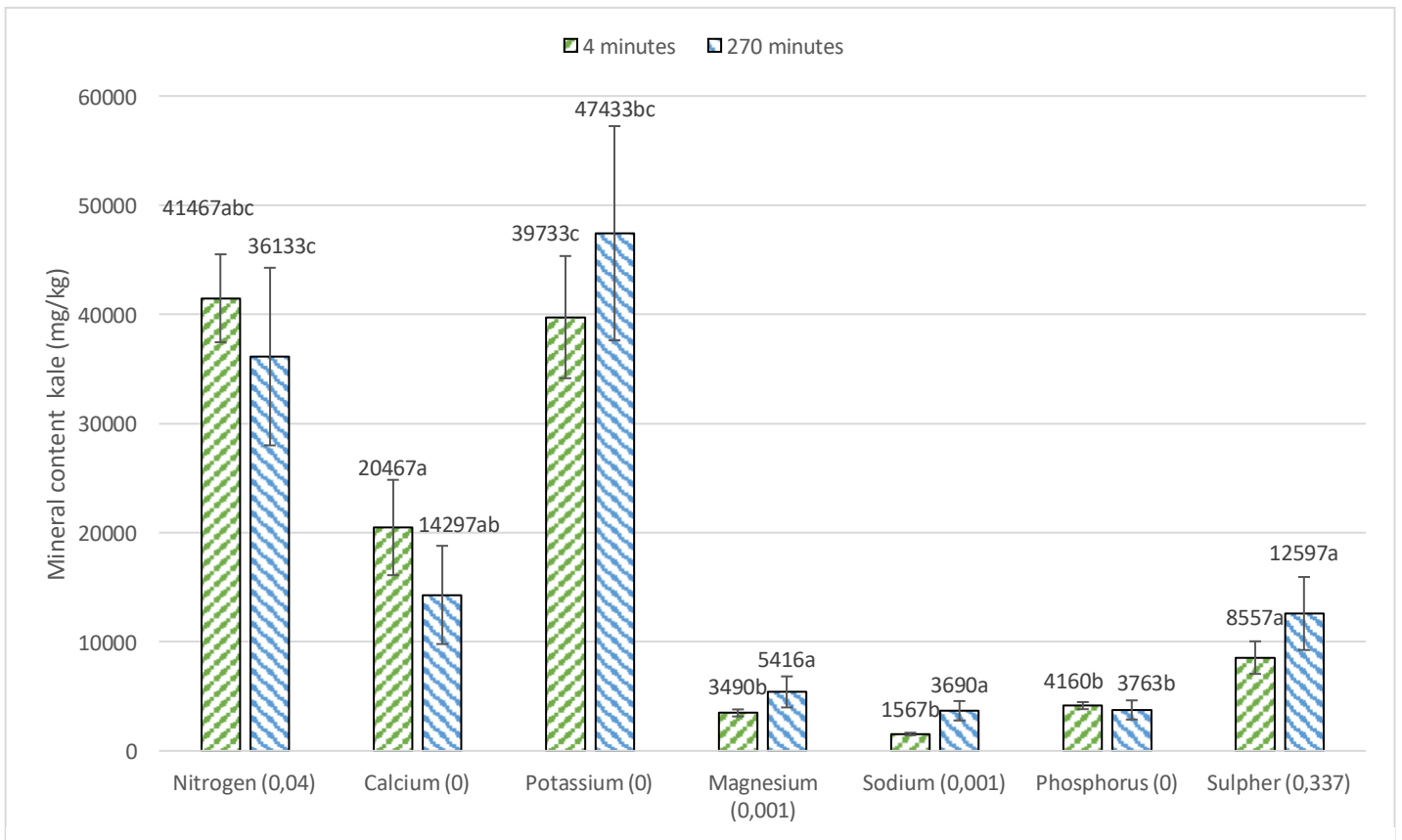


Figure 11: Mineral content given in milligram per kilogram of dry weight kale under two different ebb and flow irrigation intervals. Error bars represent standard deviation and different letters above the columns and next to data label within each element indicate a significant difference ($\alpha = 0,05$) between different irrigation intervals. Value after each element given between brackets indicate the probability of obtaining the observed results. Expressed a p-value in ANOVA.

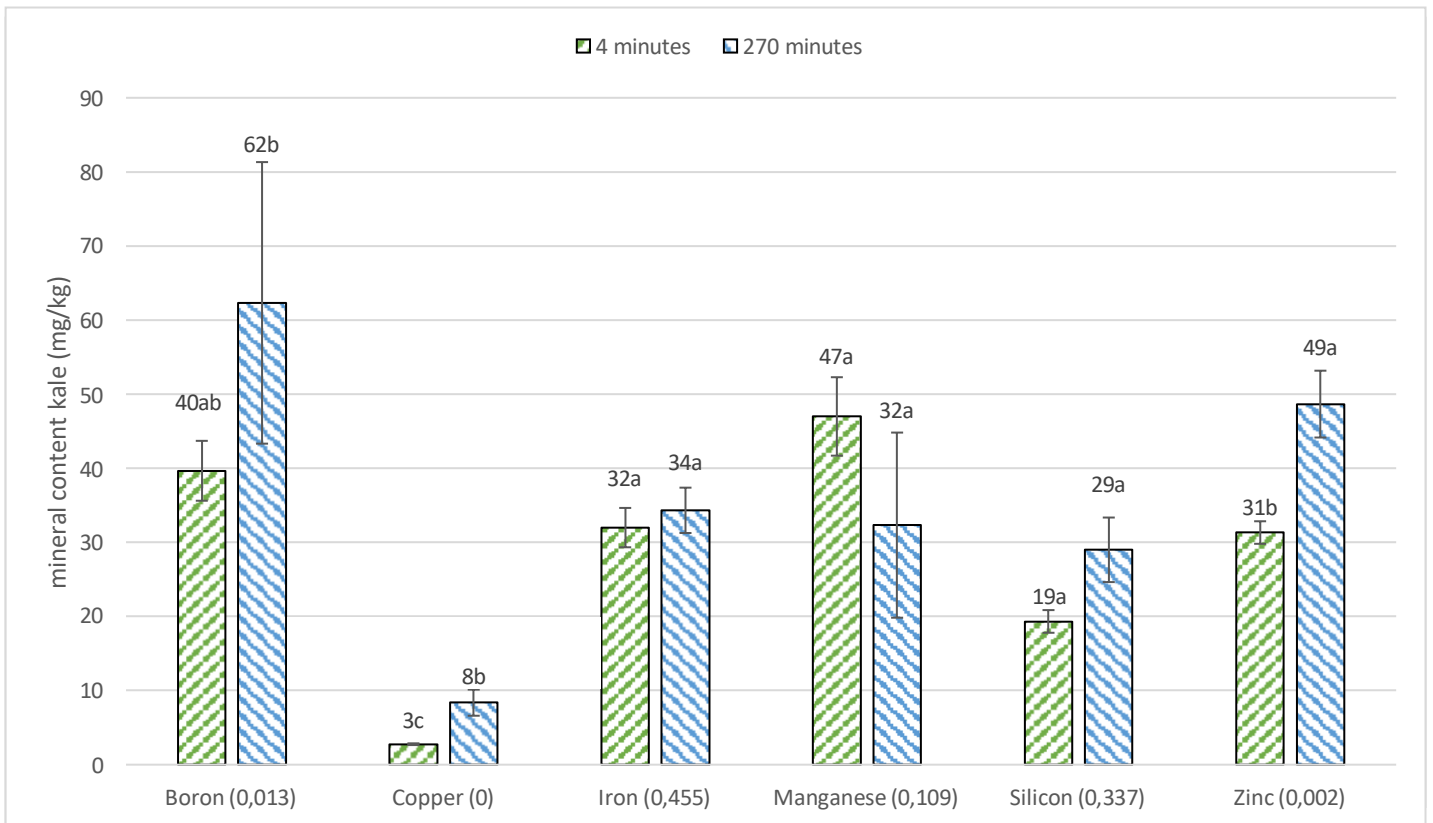


Figure 12: Mineral content given in milligram per kilogram of dry weight kale under two different ebb and flow irrigation intervals. Error bars represent standard deviation and different letters above the columns and next to data label within each element indicate a significant difference ($\alpha = 0,05$) between different irrigation intervals. Value after each element given between brackets indicate the probability of obtaining the observed results. Expressed a p-value in ANOVA.

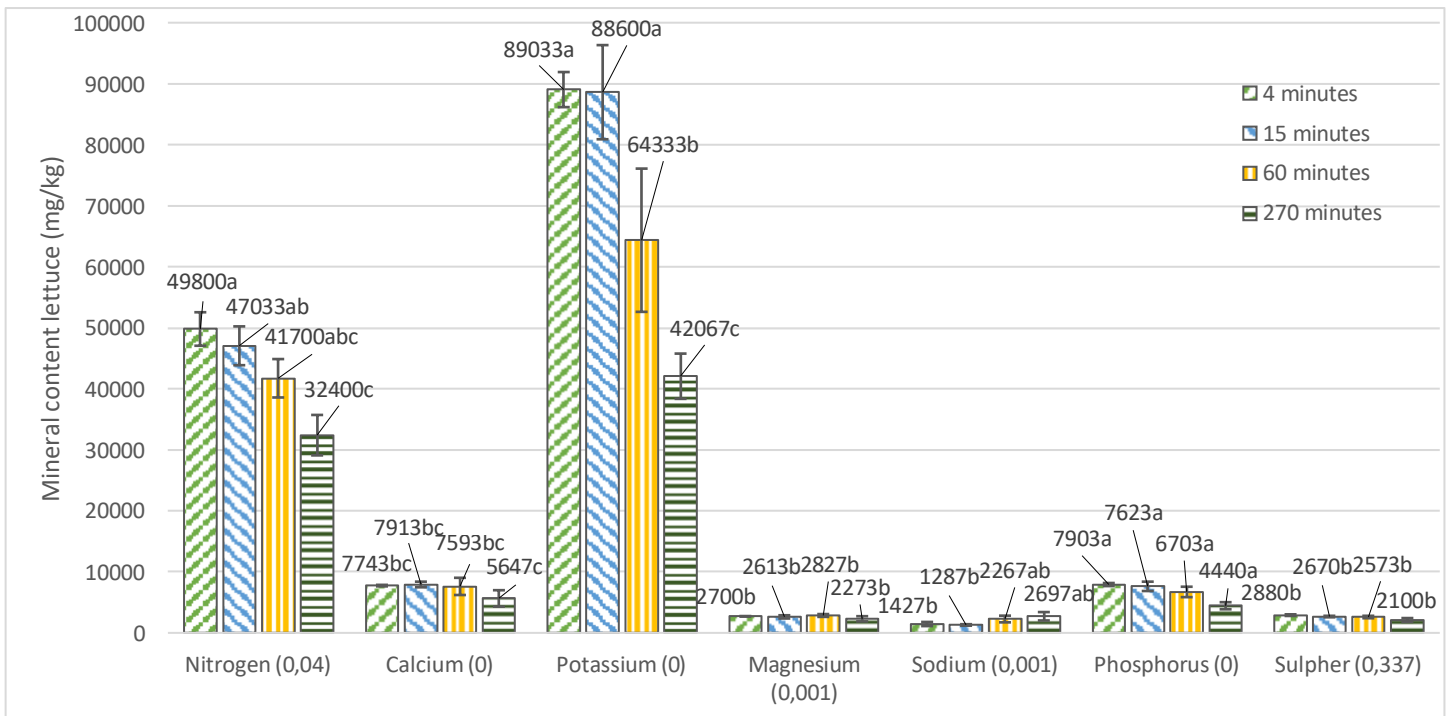


Figure 13: Mineral content given in milligram per kilogram of dry weight lettuce under four different ebb and flow irrigation intervals. Error bars represent standard deviation and different letters above the columns and next to data label within each element indicate a significant difference ($\alpha = 0,05$) between different irrigation intervals. Value after each element given between brackets indicate the probability of obtaining the observed results. Expressed a p-value in ANOVA

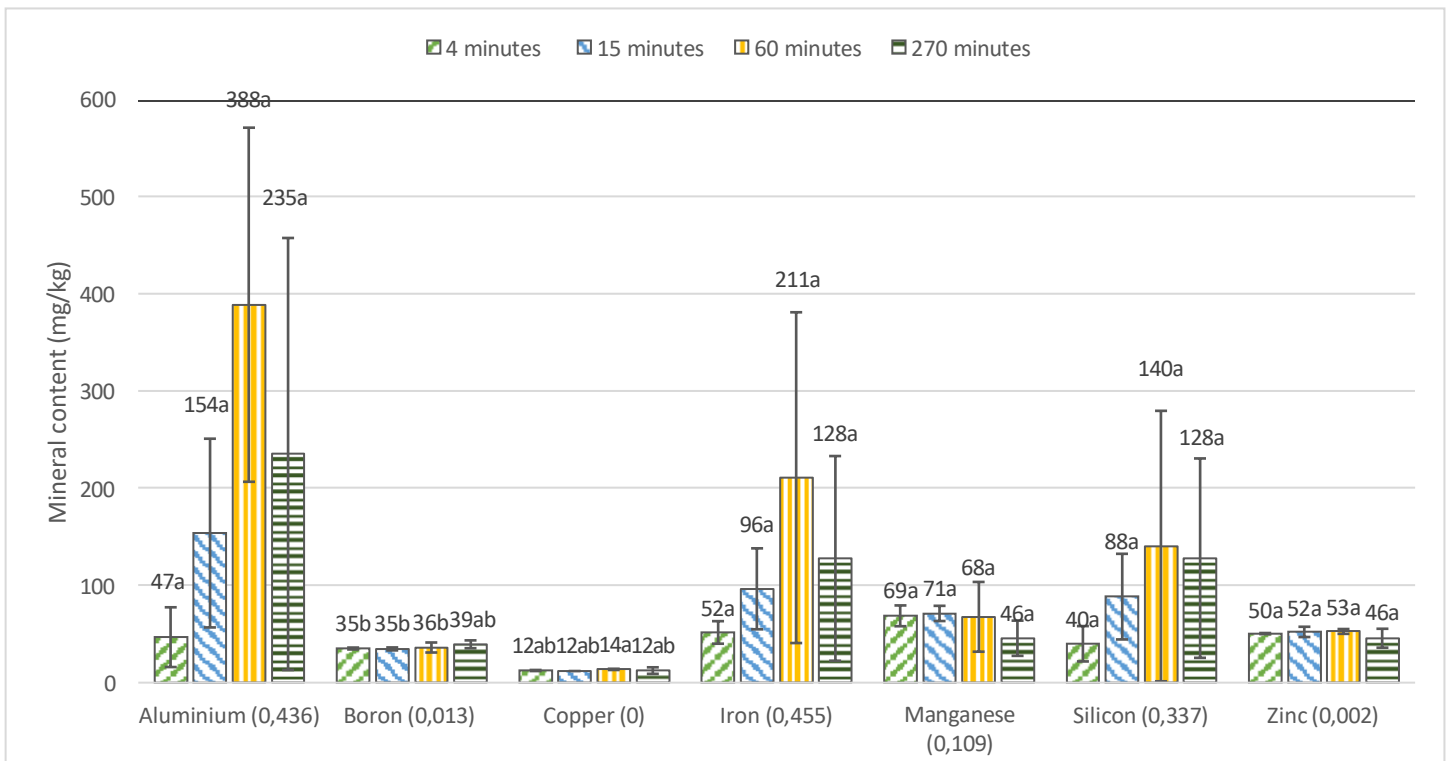


Figure 14: Mineral content given in milligram per kilogram of dry weight lettuce under four different ebb and flow irrigation intervals. Error bars represent standard deviation and different letters above the columns and next to data label within each element indicate a significant difference ($\alpha = 0,05$) between different irrigation intervals. Value after each element given between brackets indicate the probability of obtaining the observed results. Expressed a p-value in ANOVA

4. Discussion

Different indicators (photosynthetic rate, stomatal conductance, fresh weight, dry weight, mineral content) of plant performance for both lettuce and green kale were found in this study. Additionally, parameters (humidity, temperature, CO₂, airflow, and, relative moisture in the plug.) measuring the functionality of the controlled vertical farm were found.

4.1 Light and climate control

Light measurement found in this experiment was the average from the four layers. Average was taken because there were minor difference present between each of the layers. Data gathered from a quantum flux meter may differ. (Barnes et al., 1993) There it is unlikely that light has had a conclusive influence on the given results especially coming from the same manufacturer and same year of production.

Results have shown a fluctuation in environmental conditions on a weekly basis for the duration of the experiment. For all cases, airflow, temperature, CO₂, and relative humidity data were presented. (Figure 4–8) The highest relative fluctuation occurred for the airflow. Several studies show that airflow difference of 0,5 m/s significantly influences photosynthetic rate (Lee et al., 2013, Kitaya et al., 2003, Kitaya et al., 2000). Including other effects like tip burn due to local calcium deficiencies (Lee et al., 2013).

Like other parameters temperature has shown to be differ during this experiment. On occasions a difference around 2 degrees Celsius has been recorded. Temperature direct or indirectly affects photosynthetic rate. Higher temperature (up to a certain level) increases photosynthetic rate. (Zhou et al., 2022) Most studies conduct will look at primarily at extreme temperature differences. However due to a potential difference of only 2 degrees it may be neglected that this has had an significant effect on lettuce and kale (Wheeler et al., 1993). Adding to this is the uncertainty of measurement. Within a vertical farm temperature may differ locally especially within the canopies of the crops (Naranjani et al., 2022, Kozai and Niu, 2016). Hence a difference will always occur and thus the temperature difference measured in this experiment may not have influenced crop production.

The use of CO₂ enhancement in vertical farms is a highly researched area. Within parameters CO₂ enrichments under growing conditions enhances crop growth (Lamichaney et al., 2021, Thompson et al., 2017). Relative low increments, in line with differences of around 50 ppm

CO₂ levels as shown in this study, according to current knowledge have not been tested. Increment difference on the effect of lettuce on CO₂ enrichment suggest the effect of 50 ppm to be negligible (Rangaswamy et al., 2021).

Lights may have a heating effect within a VF. In this experiment when lights turn off temperature decreases. When temperature decreased humidity increased. This follows the physical properties, unrelated to vertical farming and even plant science, have explored (De Bruin et al., 1999). When air cools down water holding capacity decreases making the air more saturated thus increasing relative humidity. During both experiments rH gradually increased, this most likely related to growth of the plant and corresponding total transpiration rate. A vertical green wall and relative humidity inside a building showed a similar pattern (Ghazalli et al., 2018). Considering this experimental design it is likely to conclude that an increase in biomass corresponds to increased moisture levels. This indicates that adequate climate control, able to control the increase in moisture in the air due to transpiration, was missing in this experiment. Different moisture levels do have an influence on the plant physiology of lettuce (Tibbitts and Bottenberg, 1976). However, it is unlikely different rH had a significant effect on the results between each layer since similar climate patterns described above correspond across all the growing layers.

Fluctuation in temperature, humidity and/or CO₂, do effect plant production within a VF. What however needs to be highlighted is that within this experiment the biggest fluctuation was found to be in airflow. Increased airflow may increase overall transpiration rate of a crop. (Lee et al., 2013) This could have created different water demands for crops within same treatment. Future research is needed to analyse its exact effect on this experiment. This research would need to include extensive mapping of airflow within the VF including the effect of growing plants, and data set including more measurements. From a technical perspective airflow would have to potentially come from more than one source (Zhang et al., 2016).

Differences between E1 and E2 might have been caused by presence of other plants. The individual layers were placed inside a climate-controlled room where other plants were growing.

4.2 Moisture measurements

Increased soil moisture can be indicated by a higher voltage potential (Yu et al., 2021, van den Dool et al., 2003). Results in this study may follow a similar pattern. Prior to irrigation a lower voltage potential was found compared to 2 min after irrigation across all four layers of the VF. In theory mechanisms do work in a similar pattern compared to soil, interesting would be if one could find relative numbers and compare them. Moisture levels may be presented in percentage of saturated content, independent on what tool taken for measurements (Ali et al., 2015). A rockwool plug may be saturated 2 min after irrigation. The analog meter suggests different values within each layer. Rockwool may have different saturation points when

exposed to different levels of water over time (Choi and Shin, 2019). This, in combination with a too small sample size, made it hard to construct a saturation point from which relative data can be taken. For future research it would be worthwhile finding saturation point of rockwool growing medium using analog moisture meter. Results from an analog moisture meter may only be taken as relatives to each other within this experiment.

4.3 Photosynthetic rate and stomatal conductance

Plant physiological responds to drought stress is a decreased photosynthetic rate and stomatal conductance (Riboldi et al., 2016, Hajlaoui et al., 2022). Results may not indicate the difference in e.g. fresh and dry weight of both kale and lettuce. Results show such a level of standard deviation that it unlikely for these finding to be representative. This is of high relevance when comparing treatments, but these findings might suggest at what level the plants are performing. Kale, depending on cultivar, is estimated to have a theoretical maximum photosyntatic rate of around 20 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (Erwin and Gesick, 2017). A study from 2017 having similar growing conditions measured photosynthetic rates of around 6 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ (Lee et al., 2019). For lettuce, one study found a photosynthetic rate of 10 under similar conditions (Zhou et al., 2020). So, it could very well be that levels of both photosynthetic rate and stomatal conductance found in this study relate to actual levels of photosynthesis. Looking into the data especially for lettuce sa very high standard deviation across the entire sample size can be seen. During the experiment the leaves of the lettuce were very fragile and prone to be destroyed when not handled with care.

4.4 Fresh weight and dry weight

One could suggest, based on moisture levels recorded in this study, that lettuce would thrive well in a high saturated substrate. Increase moisture within substrate correlates with increase biomass parameters. Lettuce grown under ebb and flow conditions showed increased levels of plant performance when submerged for a longer period of time (Yang et al., 2018). Furthermore lettuce growing under a nutrient film technique (NFT) shows improved fresh and dry weight compared to cultivation within an ebb and flow system (Al-kinani et al., 2021). One could interpret NFT as increased availability to water and nutrients for longer period. Due to many factors controlled within this study (substrate, irrigation methods, and climate conditions) relatable studies cannot be presented in this paper.

Results on dry weight of kale do not indicate a similar trend as lettuce does. Relatable studies on Brassica (oleracea) to support findings of this study are rare if non existing. A soil-based test indicated that a well water Brassica plant shows higher indicators of plant performance compared to base line and deprived watering (Pan et al., 2011). Unfortunately, moisture levels in the plug cannot be related with results from this study. Still, indicators of good plant performance are found both at highest and lowest irrigation interval within this study.

Potentially base line water levels have not been found in this experiment and plants were constantly over watered.

4.5 Nutrient content

Performance of a plant may be suggested by increased or decreased levels of macro nutrients (Muratore et al., 2021, Tsukagoshi and Shinohara, 2020). All these however are soil-based research. A study on *Beta vulgaris* grown hydroponically successfully use different levels of minerals as growth indicators. Increased growth parameters correlate with increased levels of mineral content for most macro nutrients. (Baiyin et al., 2021). Reliable and trustworthy data from lettuce suggest increased plant growth under smallest interval of irrigation. After carbon nitrogen is the most abundant element present. It is part of essential parts such as, protein, nucleic acid, chlorophyll, co-enzymes, phytohormones, secondary metabolites (Marschner and Marschner, 2011). Levels of nitrogen in this study correlate with these findings, to be higher under smaller irrigation intervals, 4- and 15-min. Potassium, being the most abundant cation, is important for metabolic/synthetic reaction including the regulation of pH and osmotic potential (White, 2017, Tsukagoshi and Shinohara, 2020). Levels of potassium in lettuce under four different irrigation intervals were also highest under smallest irrigation intervals, 4 and 15 min. Both the levels of mineral nitrogen and potassium indicate that smaller intervals of irrigation within an ebb and flow system are beneficial for the performance of lettuce.

For kale no significant differences were found in macro nutrient content. Additionally, limited trustworthy data on biomass production, both dry and fresh weight was found. Even though dry weight suggests a difference between irrigation intervals it does not correspond with the entire data set. Dry weight of kale is similar under lower and high irrigation intervals. For kale only significant differences for sodium, magnesium, copper, and zinc were found. In all cases mineral levels were found to be higher under 270 min irrigation interval compared to 4 min. These micronutrients decrease when availability of water increases. It can be suggested that increased irrigation intervals lead to increased moments of anaerobic conditions. Roots activity and nutrient uptake is decreased due to a decrease in ATP availability. Generally speaking water and nutrient uptake is inhibited under these conditions (Elzenga and van Veen, 2010, Trang et al., 2010). One effect of decreased levels of zinc can be correlated to decreased plant performance as reported by many studies (Erenoglu et al., 2011, Fan et al., 2021, Yamauchi et al., 2019, Arif et al., 2022). Adding this to the equation it can be suggested that slightly more favourable conditions can be found under a decrease irrigation interval for kale. Keeping in mind that due to a limitation in resources for this experiment only two of the four different irrigation intervals were tested for mineral content. We can consider that the optimal level of irrigation interval may be present somewhere between the different irrigation intervals. Furthermore, results are hard to relate due to the lack of relevant studies. The one reliable study found had a factor 10 difference in concentration of minerals for most macro nutrients in kale (Tan et al., 2023). This might be related to plant being analysed at different stages, shoot vs more mature plants, but highlighting the difficulty and necessity of reliable studies.

4.6 Reflection on experimental set up

Differentiation between experiment 1 and 2 was confusing but essential unfortunately. Experiment 2 was a natural evolution from experiment 1. During the execution of experiment 1 fresh weight was collected but not labelled as individuals. This caused dry weight not to be relevant to the individually weighted sample. Experiment 2 included individual sampling of produce and thus was sent for analysis. Other data collection, photosynthetic rate, stomatal conductance and, soil moisture were only conducted during experiment 1 due to availability. This could have been avoided by using experiment 1 as a trail and conduct improved version as experiment 2. However due to availability of growing space within SweGreen and the limitations named above this was not feasible.

4.7 Future studies & outlook

Mineral analysis of both kale and lettuce has both relative and absolute different values. Obviously, this can be related to difference in plant species (Baiyin et al., 2021, Rao et al., 1995). Future study could look at difference in mineral content using different substrate including soil. Understandable this might seem odd since soil is not used much in hydroponics. But it is important because it may make soil data more relatable to hydroponics without repeating research previously conducted. For better understanding of optimizing moisture levels within growing mediums and make them more relatable to other studies methods may be used from previous studies (Ferrarezi et al., 2015, Nemali et al., 2007). No data on difference in mineral content distribution within the plant were taken. Active and passive transport of ions are regulated differently throughout the plant (White, 2017).

A meta-analysis of available research with an aim of how data should be share most accurately witin CEA would be beneficial. One of the strengths of CEA to more traditional agriculture is that results may be more relatable independent on e.g. location and climate. To make use of this strength as much information must be shared as possible, as suggested by and in this research. However, this necessity is not reflected by three very recent publications within the search area of ‘controlled-environmental agriculture’ (Mei et al., 2023, Islam et al., 2023, Modarelli et al., 2023). None of these three suggest including all parameters discussed in this research that may be considered. One suggestion of improvement for this is looking at the correlation of humidity and temperature. An example could be integrating vapor pressure deficit (VPD) in future CEA research (Grossiord et al., 2020). VPD may directly link relationship of temperature and relative humidity to plant performance.

Furthermore example on how to share data without losing its scientific purpose can be found where data is presented as downloadable supplementary data (Baiyin et al., 2021).

5. Conclusion

Mineral content, fresh and dry weight of lettuce under four different ebb and flow irrigation intervals suggest most optimal irrigation conditions between 5 and 15 min within this experimental set-up. This correlates with relative higher level of moisture within the growing medium. No significant difference between these two smallest irrigation intervals can be found. For kale data is inconclusive and no optimal irrigation levels have been found. It can be suggested that more optimal conditions can be found towards a larger interval of around 270 min within an ebb and flow system because of the mineral content of micro-nutrients found.

Based on the monitoring of environmental data, airflow was hardest to control. It is suggested that based on results found in this research controlling airflow will increase trustworthiness of research at SweGreen.

This scientific research should be interpreted as as relatively unique due to the lack of relatable research. Henceforth sharing information for the purpose of scientific research is vital. However due to the technological advancement of industry compared to most academia an open conflict that needs to be corrected is highlighted.

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