

Documentation of Boron Deficiency and Phytotoxicity in *Cannabis sativa* to Aid in Development of the GrowDoc Application

PROJECT REPORT *CONFIDENTIAL*

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Project Team

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Project Objective

The objective of this study was to gather photo and video documentation of *Cannabis sativa* plants as they were subjected to boron deficiency and toxicity. The photo and video documentation will be used by GrowDoc App Inc. (GrowDoc) to improve their mobile application, which will help cannabis growers better discern nutrient deficiency and phytotoxicity.

Challenge

GrowDoc App Inc. (GrowDoc) is a software company whose core product is the GrowDoc mobile application (app). GrowDoc's objective is the diagnosis and solution of any plant growth problem faced by cannabis growers. The app diagnoses issues in the plant by scanning and analyzing images with its advanced machine learning algorithm, including nutrient deficiencies and phytotoxicities. Once an answer is given, users can not only

find a solution, but also browse the rest of the diseases and deficiencies database hosted on the app. GrowDoc is a readily available solution to growers worldwide, to determine plant problems earlier and reduce the risk of flower quality and quantity loss.

GrowDoc's current model has been trained on over 40,000 images of cannabis plant issues, but of those, it does not have sufficient material on boron deficiency or phytotoxicity. Boron (B) is an essential micro element for plant growth. The most significant effect of B deficiency is the inhibition of root elongation, while excess B can induce the production of reactive oxygen species (ROS). Not having access to sufficient images pertaining to one specific issue means the model will not have enough data to train on that specific issue. This translates directly to an inability in diagnosing cannabis growth issues within the app. In essence, without enough data on a particular growth issue and the training that comes with it, GrowDoc's model will not be able to perform a correct diagnosis, which is why this project will be so useful to bridge that gap.

Solution

GrowDoc partnered with the Horticultural & Environmental Sciences Innovation Centre (HESIC) at Niagara College on a course-based research project to support development of the app. In order to gather the data needed to inform the machine learning model, GrowDoc needed to compile a substantial amount of information on a given plant issue, meaning visual material on the symptoms produced within the plant. To take these pictures, GrowDoc needed a space equipped with all the necessary tools to grow the cannabis plants in the exact conditions that would allow for a certain growth issue, such as a deficiency/phytotoxicity, to manifest itself visually. Additionally, it required an expert team to monitor said plants during their growth and ensure they were on track and not developing additional issues that might cloud the results. The HESIC team provided the space and expertise needed. The team grew the plants under specific conditions and involved course students in monitoring their growth to allow for the deficiency or phytotoxicity to manifest, in order to have been documented by photo and video.

Executive Summary

Micronutrients are essential for plant's growth, productivity, and reproduction. The following eight micronutrients are considered to be essential for higher plants: copper (Cu), nickel (Ni), molybdenum (Mo), boron (B), chlorine (Cl), zinc (Zn), manganese (Mn) and iron (Fe). In this report, findings are summarized of plant symptoms as a result of boron (B) nutrient deficiency and phytotoxicity. Findings were recorded as visual material on the symptoms produced within the plant. The visual material will be used by GrowDoc App Inc. (GrowDoc) to improve their mobile application, which will help cannabis growers better discern nutrient deficiency and phytotoxicity.

1. Introduction

Cannabis (*Cannabis sativa*) is an important horticultural crop grown for medicinal and recreational purposes. Following the 2018 repeal of cannabis prohibition in Canada, production of cannabis has become an important part of the Canadian horticulture industry (Zheng, 2021). In the cannabis community there is a still lack of scientific information about optimal growing conditions, such as supply of mineral nutrients.

Macro and micronutrients are required by plants for appropriate functioning and development. Micronutrients are vital for completion of the plant's life cycle (Ali et al., 2020). They are involved in biosynthesis of nucleic acids, protein, cofactors, carbohydrate and lipid metabolism, stress tolerance, anti-oxidative systems, chlorophyll maintenance, and electron transport (Arigony et al., 2013). The optimal access to micronutrients is critical for optimum crop nutrition and development. Boron is involved in increases of cell wall thickness and flower production, as well as germination, retention, and pollen tube elongation. It's crucial for seed and fruit development as well as drought tolerance of crops. Boron supports translocation of photosynthates and inhibits IAA oxidation (Reguera et al., 2010 & Matas et al., 2009).

Micronutrient deficiency develops in the growth media as a result of changes in the organic matter content, soil pH, adsorptive surfaces, and other biological, chemical, and physical environmental conditions. Boron deficiency develops as a result of decreased root respiration, advanced cellular transport, rise in antioxidants and ROS-scavenging proteins (Yang et al., 2013.) The plant uptake of boron will be stopped as a result of very basic pH. An excessively dry grow media or very low humidity (below 25%) can also prevent uptake of Boron. As a result of boron deficiency *C. sativa* can develop one of the following symptoms: the growing tips become abnormally thick, and growth is stunted. Brown spots may appear. As it progresses new growth will be twisted or clawed and may die off. Roots and buds will not develop properly (Cockson et al., 2019).

Micronutrient toxicity leads to various phenotypic and genotypic changes in the plants. When the internal quantity of the micronutrients surpasses the threshold, they cause phytotoxicity. The common causes of boron toxicity are as follow: for outdoor growers certain soils around the world can be very high in boron (Australia and North Africa). Some well water can also be high in boron. Improper feed calculations of any nutrient including boron can lead to phytotoxicity. As a result of boron phytotoxicity *C. sativa* can develop chlorosis or bronzing around margins of lower leaves. This will spread inwards eventually killing the leaf (Cockson et al., 2019).

2. Materials and Methods

2.1. Location

The study was conducted between January 9 and March 27, 2023, on the growth bench Lot C located in room #2 of the academic cannabis indoor growing facility (CannaBunker) at the Daniel J. Patterson campus of Niagara College (NC) in Niagara-on-the-Lake. Growing conditions within the CannaBunker were maintained at 22°C-24°C for the vegetative and early flower phases, the temperature was lowered to 20°C-23°C for mid flower and 19°C-22°C for late flower. Relative humidity (RH) was maintained between 57-67% for the vegetative and early flower phases and lowered to 57-60% for mid and late flower.

Plants were grown vegetatively, under 18/6h light/dark conditions, for three weeks before switching to a 12/12h light/dark photoperiod to induce flowering. Plants were grown under short-day conditions (flowering) for eight weeks before being harvested. The light was provided by using Lumigrow Pro 650e LED light fixtures. The Lumigrow lights allowed for adjustments of light spectrums in the white, red and blue spectrums.

For the vegetative phase, the ratios of light spectrums stayed consistent at White-100%, Blue 80%, Red 60%. For early, mid and late flower phase the lights were staggered during the first and last half hour of the lighting periods going from low to high in the morning and high to low in the evening. This was done to simulate the sunrise and sunset that the plants would experience in nature. See Table 1 for the spectrum values. Light intensity measurements at cutting canopy height were 400-500PPFD for vegetative phase, 753PPFD for early flower and 832PPFD for mid-late flower.

Table 1. Light intensity and wavelength schedule of Lumigrow Pro 650e LED lights during the flowering stages of *C. sativa*.

Phase of Growth	Lights on – 30mins into day	30 mins into day – 1 hour into day	60 mins into day to 11 hours into day	11 hours - 11:30 hours into day	11:30 hours into day – 12hr (lights off)
<i>Early and Mid Flower</i>	White-100% Blue- 50% Red-75%	White-100% Blue-60% Red-90%	White-100% Blue- 80% Red-95%	White-100% Blue-60% Red-90%	White- 100% Blue-50% Red-75%
<i>Late Flower</i>	White-100% Blue- 50% Red-75%	White-100% Blue- 65% Red-90%	White-100% Blue- 80% Red-100%	White-100% Blue- 65% Red-90%	White- 100% Blue- 50% Red-75%

2.2 Materials

All cuttings used in this trial were from *C. sativa* 'White Shark' cultivar. Clones were rooted for three weeks in rockwool cubes with NC's A and B vegetative stock mix, a complete nutrient solution. The rooting hormone Stim-Root® No.1 0.1% IBA Rooting Powder (Master Plant-Prod Inc.) was used to promote roots in this trial and Regalia® Rx Biofungicide Liquid Concentrate (Marrone Bio Innovations) was applied once for powdery mildew control, supplied by NC. Cuttings used in the trial were of the same heights (5"-6"), stem diameters, and states of health. Throughout the trial the plants were sprayed on a weekly basis with BioWorks Milstop® Foliar Fungicide at low rates for powdery mildew control.

2.3 Methodology

After two weeks of rooting, on January 9, 2023, nine cannabis plants were transferred into deep-water culture (DWC) systems. Each DWC unit used an 8 L black plastic bucket (24 cm height and 22.5 cm diameter) as the nutrient solution reservoir. Two-week old cuttings were transplanted into each DWC unit, one cutting per DWC, using a net holder (Bob's Grow Mart plastics, 7.5 cm height and 9 cm diameter) filled with clay pebbles and inserted flush to the top of the bucket lids, with the bottom one cm of the net holder submerged in the nutrient solution.

Each DWC bucket was supplied with nutrient solution and had a small air-stone (approximately 6 cm³) to continuously mix and aerate the solution. The plants were grown in a complete nutrient solution containing 1.5 mM KH₂PO₄, 2.5 mM Ca(NO₃)₂, 0.5 mM NH₄NO₃, 2.5 mM KNO₃, 1.5 mM MgSO₄. Reverse osmosis (RO) water was used to make the nutrient solutions. All plants received the same concentration of a commercial ethylenediaminetetraacetate (EDTA) and diethylenetriamine pentaacetate (DTPA) chelated micronutrient mix (Plant-Prod Chelated Micronutrient Mix; Master Plant-Prod Inc., Brampton, Ontario, Canada) containing: 0.0375 mM Fe, 0.0109 mM Mn, 0.001846 mM Zn, 0.00047 Cu, 0.00629 mM B, and 0.000188 mM Mo for one week (adjustment time). This week of complete nutrient feed was carried out to promote an even nutrient supply in order to avoid transplant shock.

On January 16, 2023, these nine plants were randomly assigned to three treatments of three plants ('replicates') each. The treatments were as follows:

- 1.) "Deficient": Nutrient solution with no boron (B) to observe the effect of boron deficiency on three *C. sativa* plants.
- 2.) "Toxic": Nutrient solution with high concentration 0.05 mM of boron (B) to observe the effect of boron phytotoxicity on three *C. sativa* plants.

3.) "Control": Nutrient solution with all nutrients supplied and the concentration of boron (B) at 0.0063 mM. This nutrient solution is used as a control in this trial. The plants exposed to boron deficiency (1) and toxicity (2) were compared to these three control plants.

These specific nutrient solutions were maintained with the same plants until the end of the trial. The initial pH of the nutrient solutions was adjusted to 5.5 with 1 M (10%) nitric acid. DWC units were topped up with pH-adjusted (to 5.6) nutrient solutions as needed. Nutrient solution pH and electrical conductivity (E.C., mS cm^{-1}) were measured using a pH/EC meter (BLU2300E Combo Metre, Bluelab Corporation, New Zealand).

2.4 Experimental design

The DWC units were placed on the bench in growth room #2 of the CannaBunker. The experimental set up followed the diagram below. Each DWC with one plant was spaced 18cm between adjacent grow units, rows and the tables edge (Figure 1).

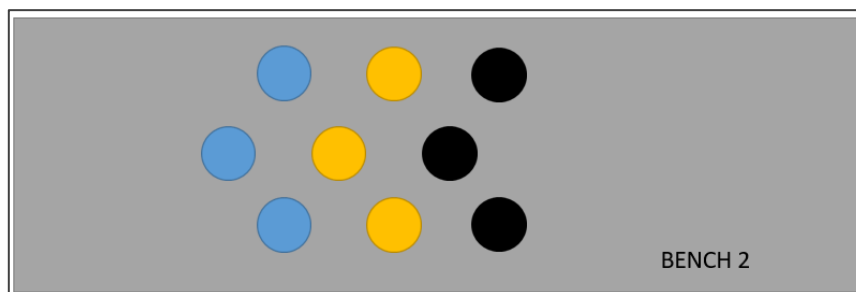


Figure 1. Diagram demonstrating the layout of the replicates, with treatments defined by colour, as grown in deep water culture (DWC) on the growing bench. Blue dots = Control (boron 0.0063 mM); Yellow dots = Deficiency (boron 0 mM); Black dots = Toxicity (0.05 mM).

2.5 Data Collection

SPAD values were taken weekly using the spectrum technologies SPAD 502 plus Chlorophyll Meter. SPAD meters are often used for non-destructive chlorophyll analysis of plants as they grow. This meter measures how effectively a leaf absorbs red and near infrared light and uses that information to infer the chlorophyll levels. Height was measured weekly using a meter stick from the lip of the net cup in the DWC unit to the apical meristem of the cannabis plant and measured in centimeters.

Weekly visual observations were collected in the form of pictures taken by researchers, students and the GrowDoc team. Each plant within the growth trial was photographically documented. Any leaves that clearly showed symptoms of deficiency and toxicity were closely photographed. Researchers also took photos of the entirety of each plant and a 360-degree video weekly. A white background was chosen for leaf close-ups to enable easier interpretation by the Artificial Intelligence (AI) program. All photos and videos collected by NC staff were shared with GrowDoc at the completion of the project.

On February 6, 2023, and March 8, 2023, samples of mature leaf tissue were collected and sent to A&L Canada Laboratories Inc. for complete nutrient analysis.

At harvest, wet (fresh) weights and dry weights of each whole plant (roots, stems, leaves, and flower buds) were measured. For each plant, the roots were separated from the plant and the net cup, weighed, and placed in the drying oven (BINDER Model FED 720) set at 30°C for seven days. Once dry, the roots were weighed again. To dry the above-ground plant parts, the plants were hung from the ceiling rafters by string, with all lights off for seven days. The room was maintained at 20°C and 50–55% RH for the first three days, then set to 18°C and 45–50% RH for the next four days.

A sample of dried buds from the top, middle, and bottom of one plant within each treatment was collected. Tetrahydrocannabinol (THC) levels were taken of these samples using the OrangePhotonics Light Lab 3 HPLC cannabinoid tester to ascertain the percentage of THC by weight at the end of the seven days of drying. After taking this THC measurement, all flower buds from all three plants within each treatment were mixed and stored, separated by treatment, for one more week. At this time, a second THC measurement of randomly selected dried buds was taken. The second THC reading was measured primarily as a learning exercise for the students of the CANN 9101 class.

3. Results

3.1 Leaf Tissue Analysis

The complete nutrient analysis of the leaf tissue collected from each treatment group clearly shows that all nutrients were in sufficient supply and were taken up by the plants as expected in all treatments (Figures 2, 3, and 4). The nutrients in the control group were all within desirable range, whereas only boron was too low in concentration (ppm) in the tissue from the deficiency treatment and too high ppm in the toxicity treatment.

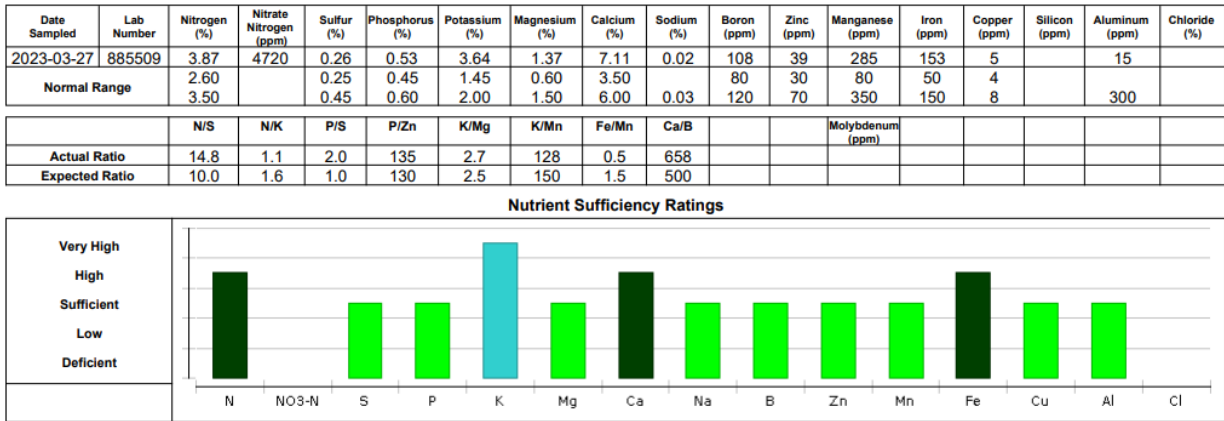


Figure 2. Complete nutrient analysis of leaf tissue from the control group at end of growth trial.

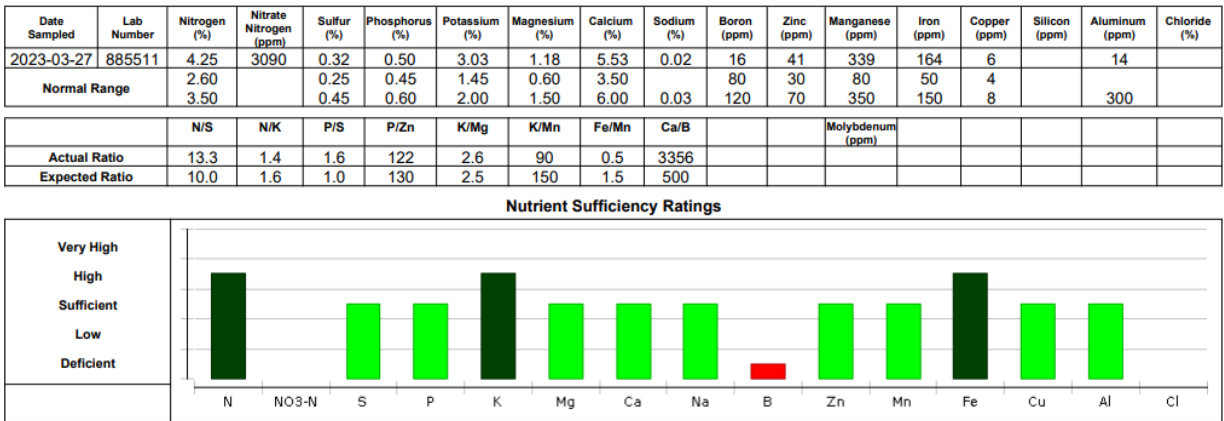


Figure 3. Complete nutrient analysis of leaf tissue from the boron deficiency group at end of growth trial.

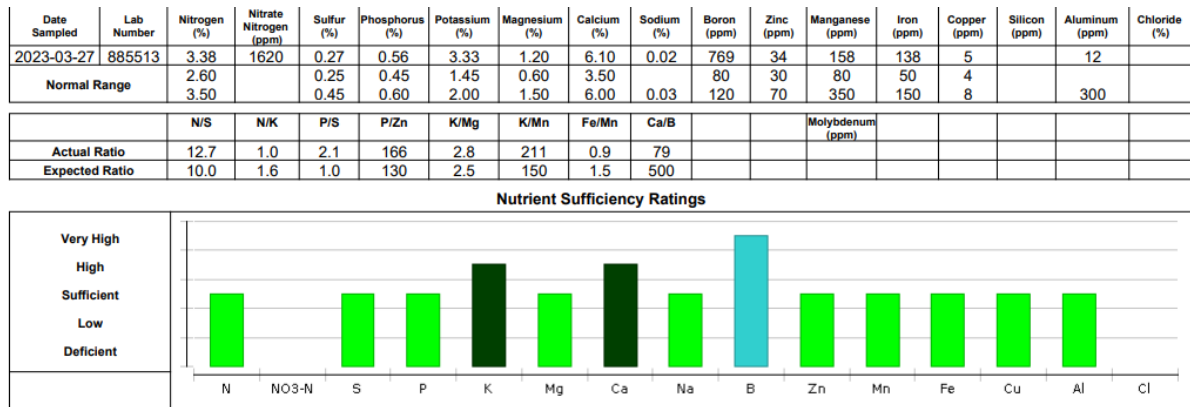


Figure 4. Complete nutrient analysis of leaf tissue from the boron toxicity group at end of growth trial.

3.2 Visual Symptoms of Boron Deficiency

The plants grown in the 0 mM Boron deficiency feed (deficiency) first developed symptoms in the 4th week (February 6, 2023) after introduction to the deficiency solution. The plants in the deficiency feed all appeared to exhibit shorter internodal spacing and the growth rate slowed compared to the control and toxicity plants, however actual measurements were not taken.

Most leaves on all three deficiency plants appeared healthy and exhibited normal growth, but it was noted by researchers that the leaf size appeared smaller than the other treatments despite being clones from the same cultivar. These were visual observations and thus were not measured. Leaves near the top of the plants did show mild chlorosis and clawing upwards but the symptoms did not spread down into the lower parts of the plant (Figure 6).

Other than appearing smaller, both in internode length and leaf size, the plants did not appear to demonstrate other deficiency symptoms to the researchers. During flowering the buds did not get nearly as large or “full” as either of the other treatments, they appeared airier and more spread out (Figure 7).



Figure 5. Visual representation of chlorosis and short internodal length observed in treatment 1 (Boron deficiency).

3.3 Visual Symptoms of Boron Toxicity

The plants grown in the 0.05 mM boron feed (toxicity) appeared to develop visual



Figure 6. Visual representation of smaller, tighter buds observed in treatment 1 (Boron deficiency).

symptoms within the first three weeks after the toxicity feed was introduced. The symptoms began with chlorosis on the leaf margins which quickly developed into a bronze necrosis (Figure 8). These symptoms affected both top and bottom leaves, affecting almost every leaf on the plant.



Figure 7. Visual representation of whole-plant (a) and up-close (b) leaf margin necrosis observed in treatment 2 (boron toxicity).

3.4 Height

Height measurements were taken weekly from the top of the net cup (base of stem) to the apical meristem of the cannabis plant. For the first three weeks after the feed change neither the deficient nor toxic boron feed plants showed a noticeable difference in their average height (Figure 8). However, after the third week the deficient boron plants were significantly shorter than the control and toxic plants. The deficient plants increased in height by less than 15 cm after week three, whereas the other two treatments continued to grow rapidly by approximately another 45 cm until week seven of measurements.

The differences in height between the three replicates within each treatment were minimal. Height of all plants in the control and toxic treatments grew to a height of 80 to 95 cm, while all replicates in the deficiency treatment grew to a maximum height of only 60 cm (Figure 9).

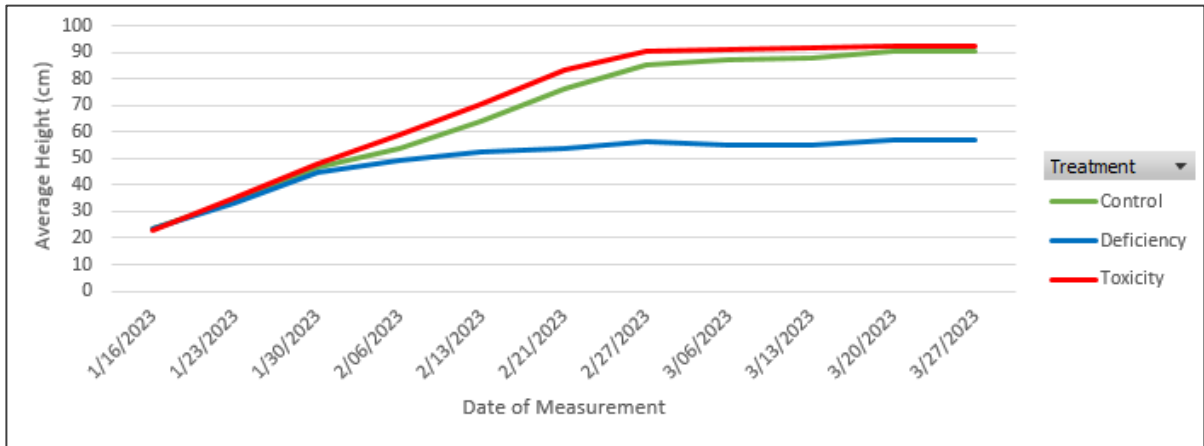


Figure 8. Average plant height (cm) of the plants grown in nutrient solutions of boron deficiency, toxicity, and adequate (control) concentrations when grown in DWC.

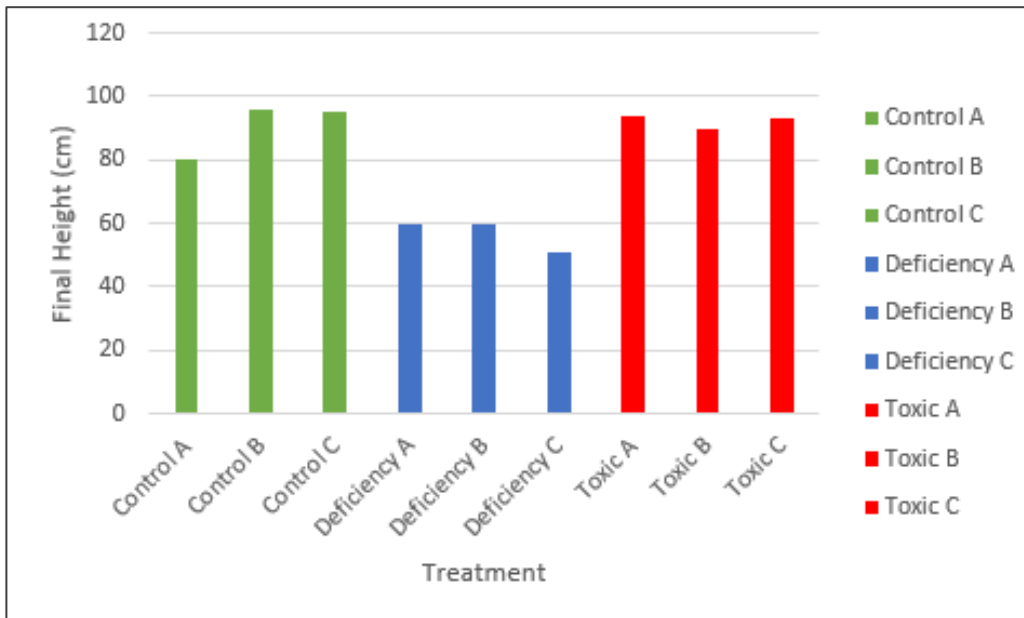


Figure 9. Plant height measurements (cm) of each plant at the end of the growth trial.

3.5 SPAD

SPAD measurements were taken weekly of all plants, three measurements were taken from mature top, middle and bottom leaves and each group of measurements were averaged to observe the total photosynthesis levels in the plants. As of February 13, 2023, two weeks after introduction of the different treatment solutions, it was clear that the control plants demonstrated consistently higher SPAD values than the deficient

treatment (Figure 10). This trend continued for the remainder of the trial. By March 13, 2023, the control had overtaken the toxic treatment as well and remained so for the rest of the trial. Although the difference is clear when looking at SPAD values it was less clear to researchers when looking at the plants, no treatment was clearly much paler or darker green.

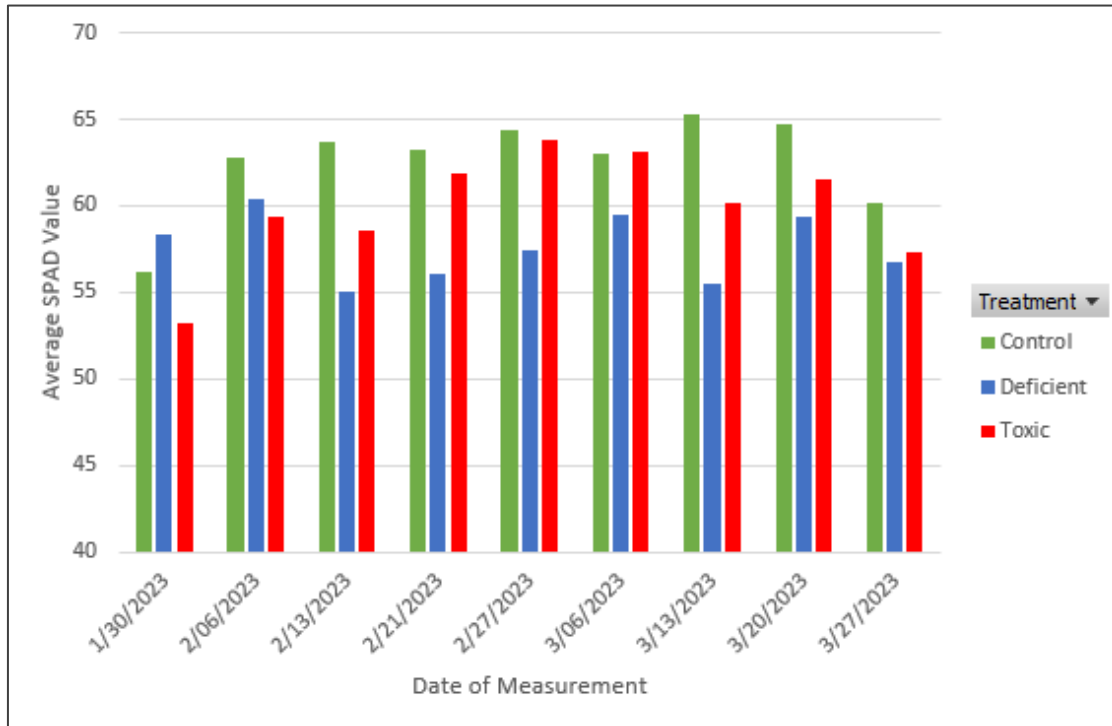


Figure 10. Average SPAD values of treatment plants throughout the growth trial period.

3.6 Wet and Dry Harvest Weights

The wet (fresh) weight for each whole plant was measured on the day of the harvest by researchers. The data showed a clear difference in weight of the deficient plants compared to the control plants, with the deficiency plants on average weighing less than half of the control and toxicity plants (Figure 11). There appeared to be no significant difference between the fresh weights of the boron toxicity plants and the control.

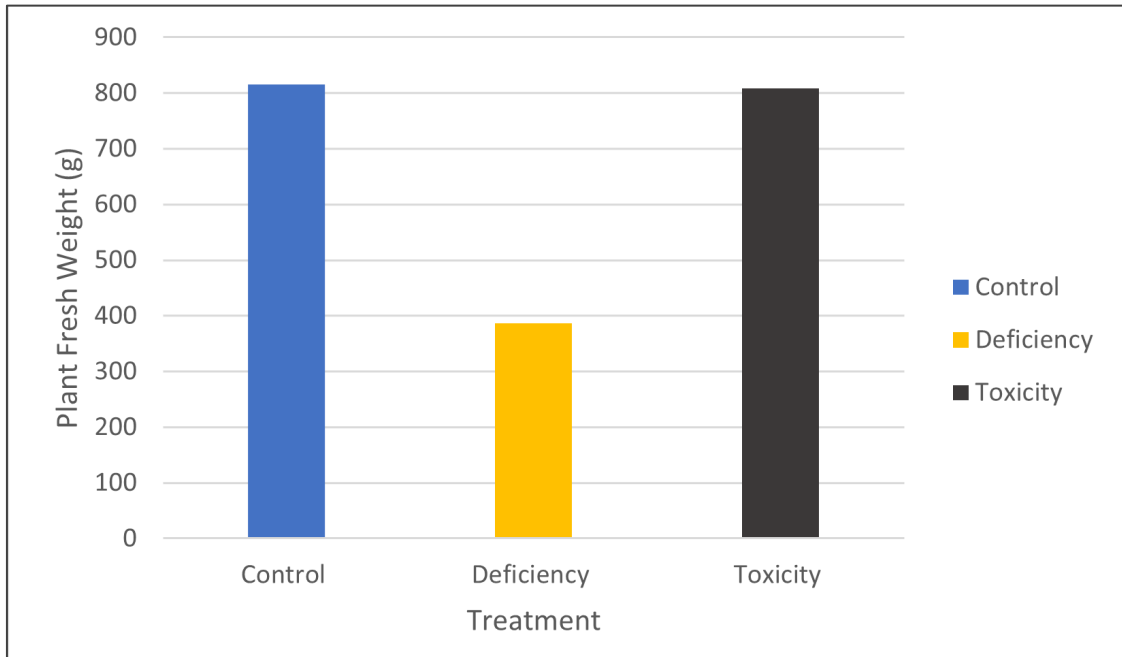


Figure 11. The average fresh plant weight (not including roots) from each treatment at harvest.

After the drying was completed plant weights were again measured and recorded as dry weight. Dried plant weight followed a similar pattern to the wet weight for each treatment (Figure 12).

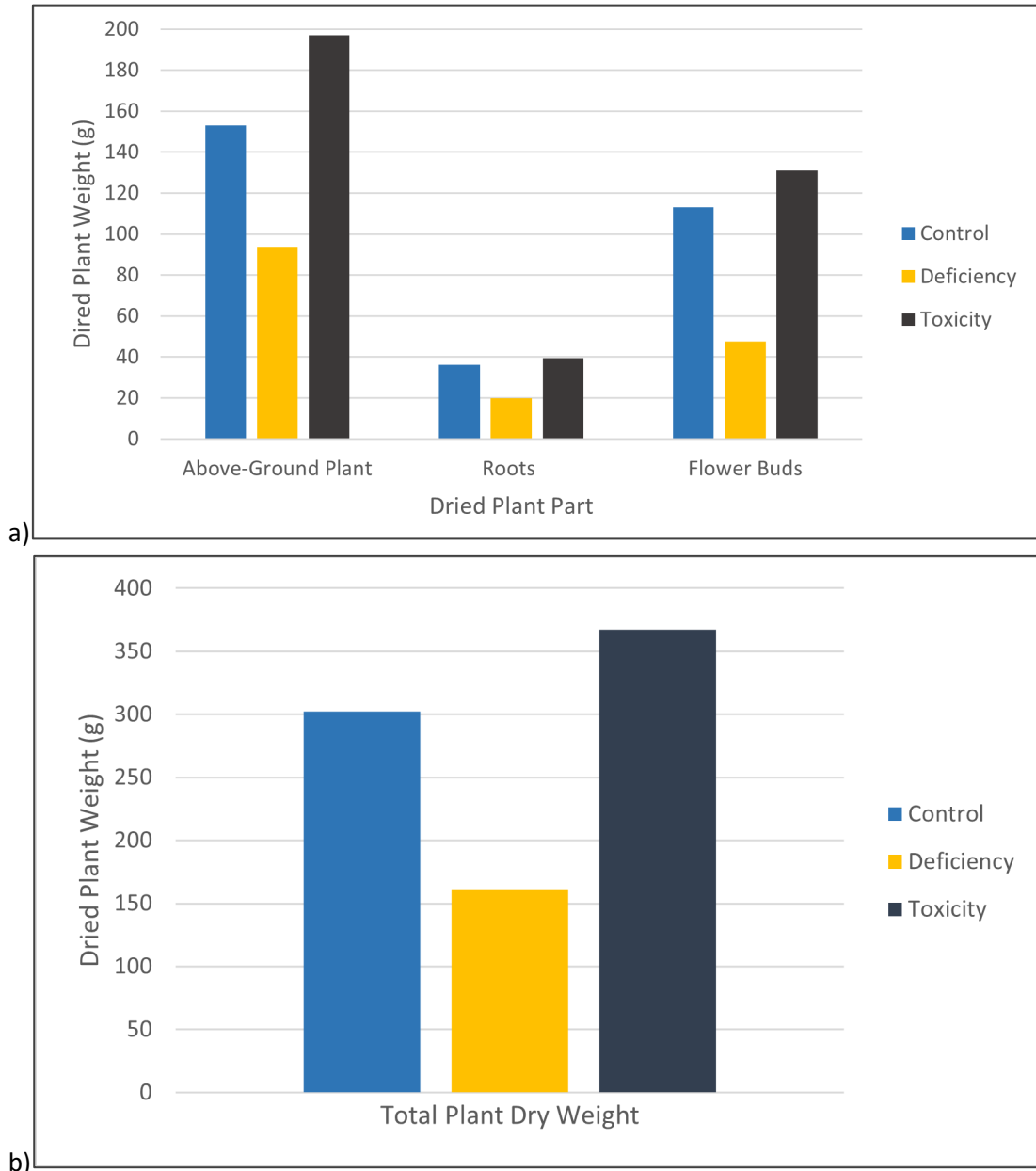


Figure 12. Dry weight average of plant parts (a) and total plant (b) from each treatment.

3.7 Bud THC levels

After drying, a representative sample of buds from each treatment was assembled using buds from the top, middle and bottom of each plant. The initial THC testing showed highest percentage in the control plants (18.3%), compared to 15.9% in the toxicity and 14.3% in the deficiency treatments (Figure 13). The second THC measurement of dried buds occurred one week after the first THC measurement. The THC percentage was

higher in all samples at this reading. The toxicity treatment increased to from 15.9% to 21.5% by weight, the deficiency increased from 14.3% to 16.9% and control had the smallest increase from 18.3% to 19%.

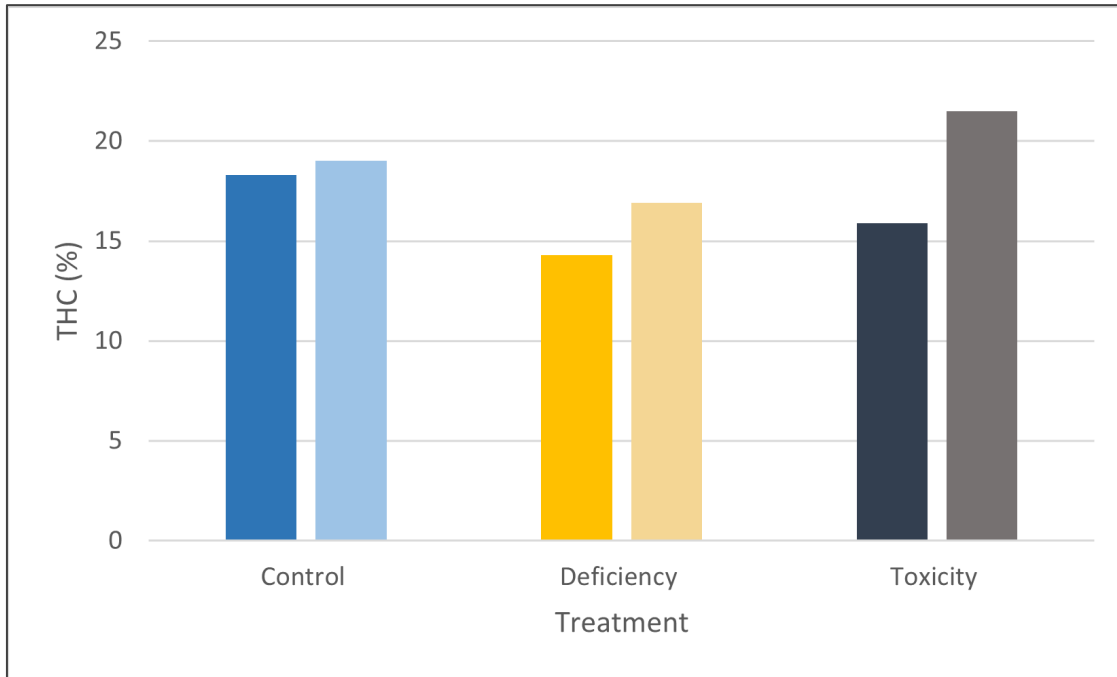


Figure 13. Percentages of THC in bud samples right after drying (darker bars) and seven days after drying (lighter shade bars) from each treatment.

4. Discussion

The boron deficient plants in this experiment produced minimal visual symptoms of deficiency. Most of the symptoms displayed were stunted growth and smaller leaves with minimal chlorosis present. This stunted growth is in line with much of the research previously carried out on boron deficiency in vascular plants, where 'boron deficiency renders decrease in cell wall plasticity leading to failure of newly divided cells to enlarge' (Ahmad et al. 2009). This lack of cell enlargement is likely what caused the differences in height, bud size and plant yield between the control plants and the plants grown in boron deficient solution. Noting these differences, if a grower were either testing a new cultivar or growing an entire crop in a boron deficient solution, it would be difficult to diagnose the deficiency during the vegetative phase. Typically, harvest quality of a crop of cannabis concerns yields and compounds within the bud, which is where largest qualitative decline was observed in the deficient plants. The deficient plants had a 41% decrease in dry bud weight and a 2.1% reduction in THC percentage compared to the

control, and the buds themselves were noted by the researchers to be more tightly compacted.

The plants grown in boron toxicity solution in this experiment showed symptoms slightly earlier in the trial than the plants grown in the deficiency solution. The earliest observations were leaf margin chlorosis developing into a bronze necrosis and continuing throughout the entire grow period. This was the only observed effect of boron toxicity on the cannabis plants since height, SPAD, yield, and THC percentages were very close or very slightly better than the control. By looking only at the growth and yield data it could be concluded that a boron toxicity could be beneficial to grower leading to a higher dry bud yield and THC levels, however having plants with so many necrotic leaves run a higher risk of disease susceptibility.

George Agrios stated in his textbook *Plant Pathology*, 'All bacteria, most fungi, some viruses and all viroids can enter plants through various types of wounds.' (Agrios, 2005). Although in the data the boron toxicity had little negative effect and some positive effects on the grow data the necrotic leaf margins throughout the plant would provide many entry points for diseases of all kinds. Disease did not occur during this trial however in a larger commercial facility the risk would be significant, and resources spent on disease management. Another issue would be the constant presence of damage on the plants would make it more challenging to scout for insect and disease pressure and damage, and other potential nutrient deficiencies.

5. Conclusions

This trial showed clear effects from both boron deficiency and boron toxicity solutions on *C. sativa* plants. Similar studies have shown similar results of boron toxicity symptoms, with necrotic edging on leaves and little effect on final size and yield (Cockson et al., 2019, Ahmad et al. 2009). However, this study did not observe twisting and malformation of the apical meristem and new leaves, which is commonly referred to as a sign of boron deficiency across multiple plant types. It is not immediately clear as to why this symptom did not show. This could potentially be a result of the strain ('White Shark') used for the experiment, which may be more resistant to boron deficiency, or possibly demonstrates boron deficiency differently from other strains. From this study, it is suggested that boron deficiency be further explored in various strains of *C. sativa* to develop a more comprehensive library of symptoms.

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