Comparison of *Arabidopsis thaliana* Strains Exposed to Heavy Metals: Applied to Agriculture

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ABSTRACT

This study reports the resistance to chromium sulfate ($Cr_2(SO_4)_3$), nickel sulfate ($NiSO_4$) and copper sulfate ($CuSO_4$) in the Col-0, Lov-1, Ws-2 and Santa Clara strains of *Arabidopsis thaliana.* Previous reports have shown illness-associated with consuming food grown in environments contaminated with such heavy metals (Shams, et al., 2018). Plant growth and biomass are also affected by considerable amounts of heavy metals in soil. To investigate the effects of heavy metals on *Arabidopsis thaliana*, plants were exposed to the heavy metals every two days, after 14 days of growth under normal conditions. Plant height was tallied daily and spectrophotometry was performed. BLAST was conducted to locate characteric similarities/differences between the genome sequences of the strains. Based on phenotypic observations, copper, nickel and chromium sulfate were found to have no significant effect on the plants, in comparison with the control. Interestingly, WS-2 exhibited excessive growth when exposed to nickel sulfate, reaching 125mm on Day 25. Only after exposing the plants to the heavy metals for the fourth time, plants exhibited wilting and leaf browning. However, statistical analysis [p result = 0.048 (<0.05)] indicated that only Col-0 was resistant to nickel sulfate. Significant differences in nucleotide strings of Col-0 include F-Box protein, Homeobox Leucine Zipper protein family (HD Zip), YABBY protein family, radical fringe and SAT gene. The elucidation of a heavy metal-resistant gene in *Arabidopsis thaliana* could potentially lead to genetic engineering of genes to cultivate heavy metal resistant plants that are safe for human and animal ingestion.

Introduction

1.1 Climate Change

1.2 Heavy Metal Impact on Environment

As a result of industrial evolution, large areas of land are progressively populated with heavy metals. Heavy metals are considered a naturally occurring element with density and atomic weight that is five times greater than water (Masindi, V., Muedi, K. L., 2018). Many of these metals may be toxic at very low concentrations. Traces of heavy metals are present in natural waters caused from industries discarding their wastes into these fresh waters (Herawati, N., et al, 1998). Common heavy metals found in contaminated soils are lead, chromium, arsenic, zinc, cadmium, copper, mercury and nickel (Wuana, R., Okieimen, F., 2011).

1.2 Heavy Metal Effect on Plants

Heavy metals are considered nonessential, which makes the exposure highly toxic for plants (Michalack, 2006). Plants that are commonly found in polluted areas may be contaminated with heavy metals in the atmosphere. Metals such as

$Cr_2(SO_4)_3$, $NiSO_4$, $CuSO_4$ are shown to affect the growth and biomass of plants such as in the plant *Arabidopsis thaliana* (Li, W., et al, 2005). Heavy metals interfere with the physiological processes of a plant, hindering their respiration, photosynthesis and elongation of the cells (Zornoza, P., et al, 2012).

1.3 Metal/Cation Exchange

Plants possess characteristics and mechanisms that grant the essential metal ions access to cellular compartments, while minimizing the damage that will be created by the nonessential ones (Michalak, 2006 ; Keilig, K., Ludwig-Muller, J., 2009). The free metal ion concentration in the cytosol is needed to be kept low in metal sensitive plants like *Arabidopsis thaliana* in order to maintain functional cell metabolism and function (Cho, M., et al, 2003). Cation exchange is an exchange of minerals between soil and roots of plants. The negative soil particles bond with cations and the cations are absorbed by the roots of plants (Urry, L. A., 2017). (Figure 1) Major minerals and particles exchanged through Cation Exchange are Potassium (K), Calcium (Ca), and Sodium (Na). The exchange of K, Na and Ca can be disrupted by heavy metals. Soil absorbs heavy metals, such as Pb and Zn, better than minerals such as Ca. Plants need required minerals and particles to grow properly (Bolanle-Ojo, et al, 2014).
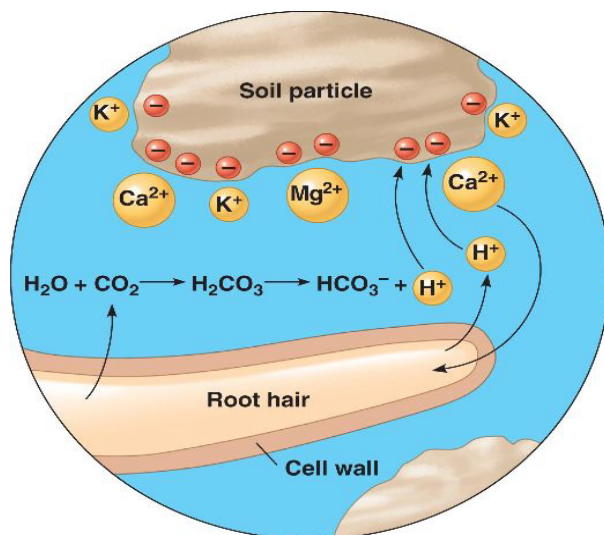

Figure 1: Mineral absorption through Cation Exchange. The roots of plants acidify the soil solution and release CO2 through respiration while pumping H+ into soil. The CO2 reacts with water, and forms H2CO3 which releases H+. The H+ ions release mineral cations into the soil through neutralization of negative soil particles. The roots absorb released cations for nutrients. Soil particles are negative, allowing nutrient Cations (K+) to bond. http://bio1152.nicerweb.com/Locked/media/ch37/soil_availability.html

1.4 Contaminated Soil Effect on Humans

Soil contaminated by heavy metals are capable of causing damage to human and animal health, and food quality of plants grown in contaminated environments (Shams, Ekinci, et al , 2018). Heavy metal absorption increases chances of humans consuming toxins via the food chain. The movement of the heavy metals through the food chain poses a threat to human health and the ecosystem (Bolanle-Ojo, T. O., et al , 2014). If the metal is a carcinogen, the carcinogenic effect can affect the plants and possibly the people eating them. In Taiwan, there was a higher incidence of oral

cancer between 1982-2002, due to the large amount of factories which release pollutants which can affect the soil. Therefore affecting the plants which grow in the area and affecting the people who consume such plants (Su, C.-C., et al, 2010).
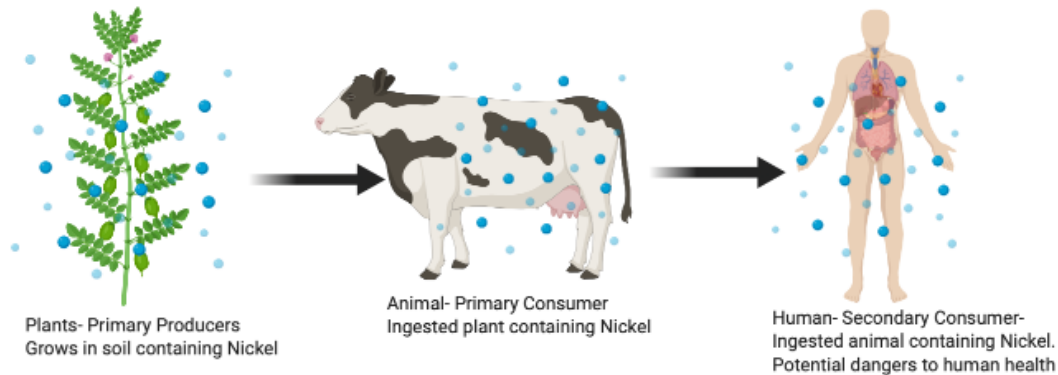
Figure 2: Food Chain. Heavy metals are not removed or lost through levels of the food chain but are conserved and passed through levels. Eventually this can become a hazard in food consumption for humans (created by Rachel Hanan, adapted from: (Su, C.-C., et al, 2010).

1.5 Genotypic Modifications

Genetic modifications facilitate the introduction, removal and tweaking of a specific gene to create a desired trait faster compared to traditional breeding. For example, transformation during genetic engineering permits introduction of new proteins or mutants that may enhance the resistance against plant pathogens (Dong, O. X., Ronald, P. C., 2019). Testing common genotypes that grow naturally may determine genotypes that present the suitable characteristics to resist exposure from heavy metals. Genetically modifying the genotype of a certain plant that grows in domains that are affected by heavy metals with a mutant of a resisting plant may allow for survival and/or increase their growth rates (Wilke, C., 2019). Wilke (2019) stated that a study was performed where GAPC1, OCT4 and GSTF1 genes of *P. vittata* were placed into plants to prevent death when exposed to arsenic. Ferns were already being used to draw arsenic out of soil in some contaminated areas. These ferns absorbed about half of the total arsenic in heavily contaminated soil over five years. Putting the genes of these specific ferns into other plants made it possible for cold-tolerant species to remove arsenic.

1.6 Purpose

Our study focused on the resistance of four strains (Col-0, Lov-1, Ws-2 and Santa Clara) of *Arabidopsis thaliana* when exposed to chromium sulfate ($Cr_2(SO_4)_3$), nickel sulfate ($NiSO_4$) and copper sulfate ($CuSO_4$). We hypothesized that there is a certain strain of *Arabidopsis thaliana* that is more resistant to one of the given heavy metals. This specific strain consists of a unique genetic pathway/protein that allows the organism to prevail under environmental stress. Col-0 is considered the wildtype or normal *Arabidopsis* genotype. Col-0 is found in wild environments, which generally contain many stressors, which it is capable of overcoming in order to grow, therefore we believe that this genotype will be more resistant than the other three. In addition, $NiSO_4$ is known to be more harmful to humans than $Cr_2(SO_4)_3$ and $CuSO_4$, so we can infer that $NiSO_4$ will be most detrimental for the plants.

Materials and Methods

2.1 Plant Growth

Seeds of four strains of *Arabidopsis thaliana,* Col-0, Lov-1, Ws-2 and Santa Clara were gifted by the Arabidopsis Biological Resource Center. The Col-0 (Columbia) wildtype is considered the *Arabidopsis* genotype or the wild-type. The Lov-1 (Lovvik) strain naturally grows in cold climates, particularly in Northern Sweden. Ws-2 (Wassilewskija) typically grows in Wassilewskija, Russia, which is cold seasonally. The Santa Clara genotype is found near San Jose, in Santa Clara County, California, and is known to accumulate a substantial amount of nickel in its cells (Arabidopsis Biological Resource Center). Moistened seeds of each strain (15-20) were planted in 2.5 by 2.5 pots containing 78.8 g of soil and 6.7 g of fertilizer (14% nitrogen, 14% phosphate, and 14% potassium). The plants were placed in respective trays filled with 1 ⅕ cm of water, then received a cold treatment at 7°C for 3.5 days. The trays were then transferred 15 cm under T5 Fluorescent grow lights for the 3 day germination period. The water level in each tray was kept at 2 cm in a 20-22°C atmosphere. Approximately 7 days after the first leaves sprouted, each pot was watered daily with 5 mL of water using a sterile medicine dropper.

2.2 Arabidopsis thaliana Exposure to Heavy Metals

The mediums contained 16 mM $Cr_2(SO_4)_3$, 16 mM $NiSO_4$ and 16 mM $CuSO_4$ all in a separate 1000 mL water solution. Starting from Day 17 of growth, all the designated pots in the tray were given 15.5 mL of their specific heavy metal mixture three times within one week. The control consisted of 15.5 mL of water. A total of five applications of the heavy metal solution was administered. After the time period that the plants were exposed to the heavy metals, each pot was watered with 5 mL of water daily.

2.3 Leaf Length Analysis

Height of leaves from each pot were measured daily. The length of the plants was recorded everyday to show a change of growth over time.

2.4 Spectrophotometric Analysis

Spectrophotometry was performed to identify chlorophyll/carotenoid levels in each plant to determine the effects of heavy metals on their biomass. To prepare the plants for Spectrophotometry, leaves ranging from 10-20 mm were crushed using a mortar and pestle. 3 mL of 80% acetone was added and the mixtures were then placed into cuvettes (12.5 x 12.5 mm) and covered. Cuvettes were placed into a Thermo Spectronic® 20 Genesys® Spectrophotometer.Wavelengths were determined by the type of chlorophyll being tested. Chlorophyll A used 680 nm, Chlorophyll B used 450 nm and Carotenoids used 510 nm. C measured concentration and A measured absorption. This was performed for each plant type and the absorption/concentration levels were recorded. If the concentration levels of the experimental were greater than the control, the inference that the heavy metals affected the plants in a positive way can be made.

2.5 Statistical Analysis

A Chi Squared test was conducted to show which strains were significantly impacted positively or negatively by any of the heavy metals. The formula that was used was . There was 2 degrees of freedom, $Pr[X>5.99] \approx 0.05$. After, a one tailed Student's T Test was used to determine the significance between the experimental groups and control at a P

value of 0.05. If the P result is less than 0.05, we can infer that the heavy metal had a significant impact on the growth or biomass of the plant.

$$\chi^2 = \sum \frac{(o-e)^2}{e}$$

2.6 Genome Analysis

Nucleotide BLAST analysis was performed between Col-0 and Lov-1, and Col-0 and Ws-2. This was performed to determine significant differences between the strains, which may have contributed to the difference in response to heavy metal stressors. The difference in nucleotide strings which code for essential genes and proteins may be the cause of the different amounts of resistance to the heavy metals.

Results and Discussion

3.1 Col-0 Height and Health

Height of all Col-0 plants was monitored. Growth was observed throughout the experiment. Height data of the plants showed that the genotypes Col-0 had shown a significant difference in effect of heavy metals in comparison to the control.

The Chi Square test was conducted and a result for Col-0 was 119.3 (>5.99≈0.05), stating that Col-0 was greatly affected. After, a Student's T Test was conducted to show which heavy metal affected the Col-0 strain the most. Results shown that Col-0 was the most resistance to the nickel sulfate than any other strain (Lov-1, Ws-2 and Santa Clara). In addition, Col-0 was most resistance to nickel sulfate than copper and chromium sulfate.

The length of the plants of Col-0 that was exposed to nickel sulfate averaged 98.3 mm in comparison to the Col-0 control of 27.3 mm. Under Copper Sulfate, Col-0 grew to an average of 14 mm and under Chromium Sulfate, grew to 29.33 mm.

Day 29 growth of Col-0, Nickel Sulfate had a p value = 0.113 (>0.05), Day 35 p result = 0.048 (<0.05). The P result had decreased from Day 29 to Day 35 showing that there is a significant correlation in the effect of nickel sulfate on the Col-0 genotype.
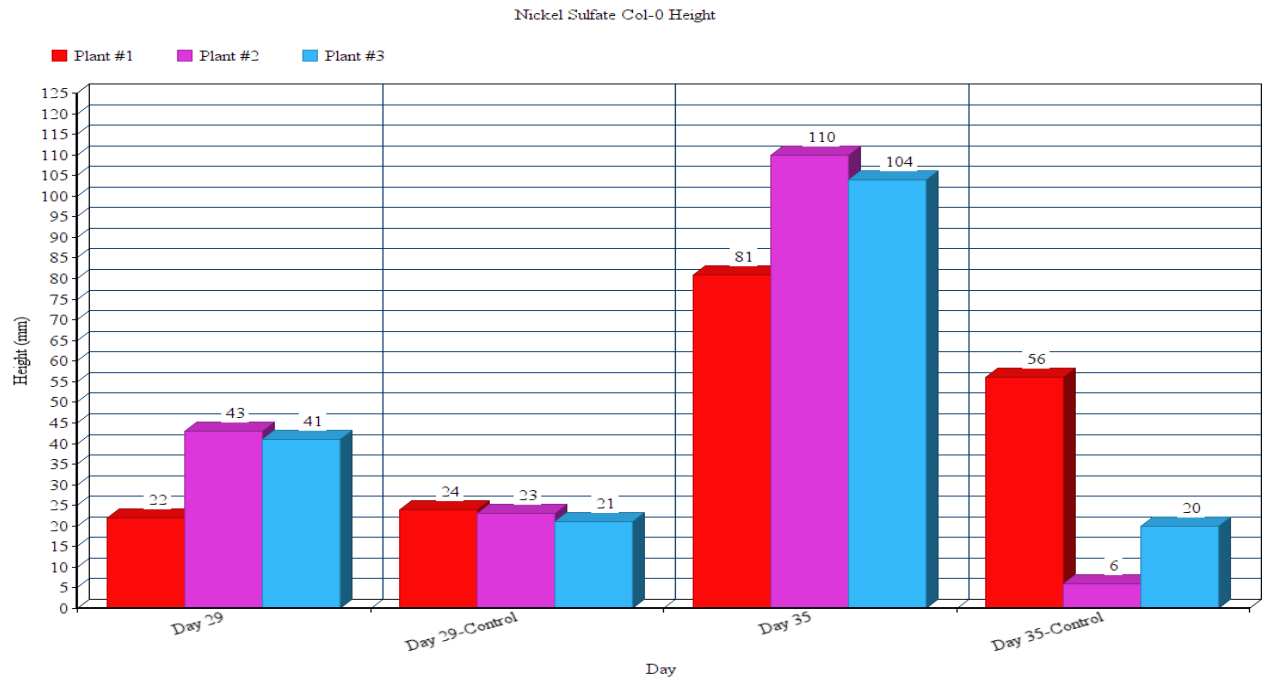
Graph 1: Col-0 exposed to nickel sulfate, growth on Day 29 & Day 35. The control and experimental of day 29 did not show a significant difference in growth level. However, the control and experimental on day 35 showed statistical significance. Showing that the Col-0 strain was resistance to the nickel sulfate and even grew better than the control.
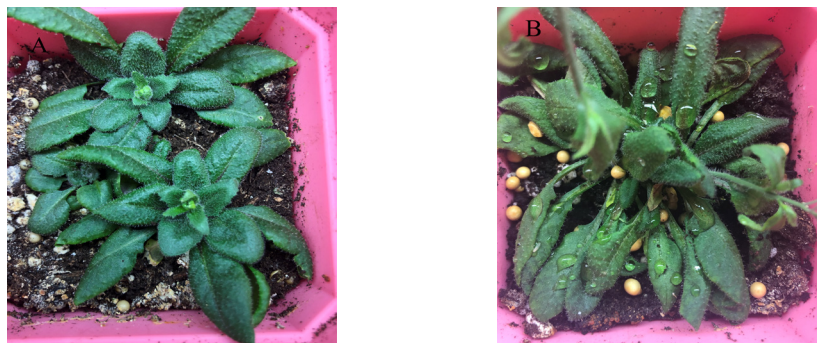
Figure 3: Col-0 under Nickel Sulfate treatment. A) Col-0 on day 29 showed a p value = 0.113 (>0.05), showing no significance. B) Col-0 on day 35 shows a p result = 0.048 (<0.05), showing a significant impact.
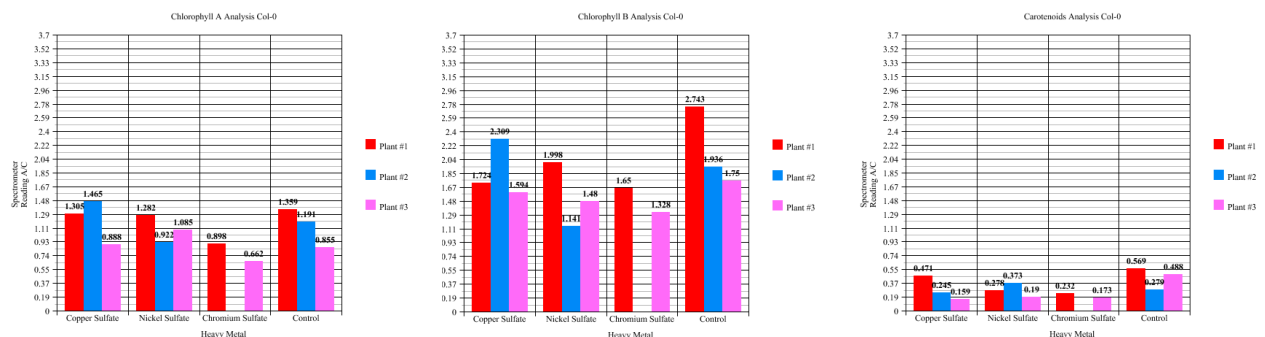
Although the T test for height of plant has shown that Col-0 was most resistant to nickel sulfate, the results of the spectrophotometry T test, Chlorophyll B (Col-0, Nickel Sulfate) had a p result = 0.035 (<0.05). Even Though, the p result <0.05, the control had an average of 2.14 A while col-0 had an average of 1.54 A. The height of col-0 plant was increasingly larger, but the health of the plant was affected in a negative aspect. P result for Col-0, nickel of Chlorophyll A = 0.408 (>0.05), p result for carotenoids = 0.165 (>0.05). Only Chlorophyll B showed a negative effect to nickel sulfate in comparison to the Col-0 control.
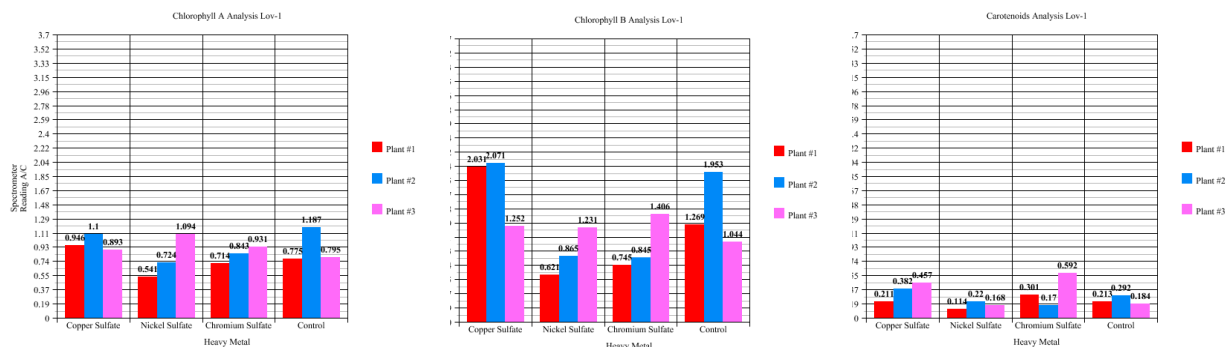
Graph 2: Chlorophyll A and B and Carotenoid levels in Col-0 strain. Chlorophyll A Graph showed no distinctive results between the control and experimentals. Chlorophyll B graph shows that in Plant 1 of the control the numbers were much greater than the rest of the control and experimental. However, T Test had shown the p result for the plants exposed to nickel sulfate <0.05, indicating a significant effect. Carotenoid analysis showed no significant differences to consider between the control and experimental.

3.2 Lov-1 Height and Health

Height of all Lov-1 plants were monitored every day. The Chi Square test was conducted for the Lov-1 strain [directional value 5.99 (0.05)]. Lov-1 result = 6.5 >5.99, which shows that there is a significant difference between the experimentals and controls. Although Lov-1 showed significance, in comparison to the result of Col-0 (119.3), it was not an extensive impact.

After, a Student's T Test was conducted to show heavy metal affected the strain in a beneficial or negative way. Day 29 growth had shown that Lov-1 exposed to copper sulfate (p result = 0.035) and Lov-1 exposed to chromium (p result = 0.037) were <0.05. The growth averages for the Lov-1 exposed to copper (4.4 mm) and chromium (3.4 mm) were remarkably lower than the control (13.4 mm). The Lov-1 strain was not resistant to the metals, in fact, the plants had shown a negative effect. Day 35, non of the Lov-1 plants of any metals had shown significance statistically (<0.05).

Student T tests were conducted on the spectrophotometry results of Lov-l, chlorophyll A and B and carotenoids. The results were >0.05, showing that there was no positive or negative significant effect of the metals on the plants.

Graph 3: Chlorophyll A and B and Carotenoid levels in Lov-1 strain. Chlorophyll A Graph showed no distinctive results between the control and experimentals. Chlorophyll B graph indicates that copper sulfate affected the health of two of the plants in a positive way. However, a T Test had shown no significant effect between the control and copper sulfate. The carotenoid analysis showed no sign of effect in contrast to control.

3.3 Ws-2 Height and Health

Ws-2 had grown at a faster rate compared to the other strain, however, this was not significant in comparison to the control, who grew to similar height. Ws-2 generally grows to 200-250 millimeters. After the 4th infusion of heavy metals, all Ws-2 plants began to wilt.
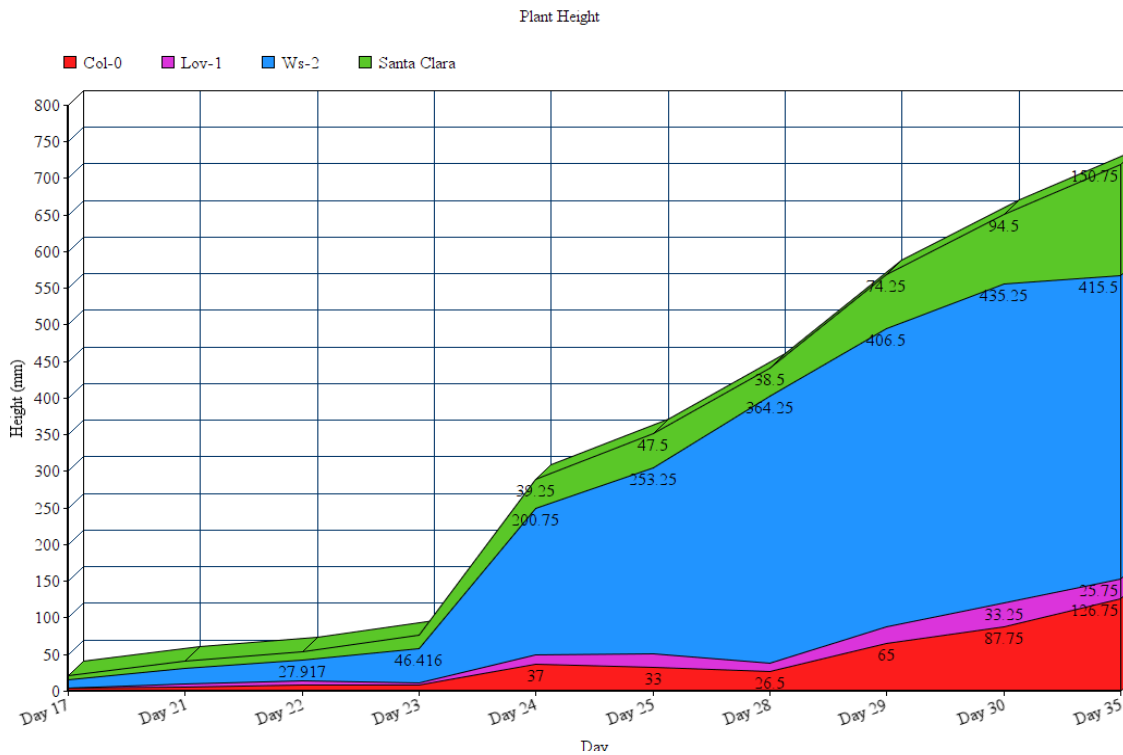
Graph 4: Height Over Course of Days. Ws-2 grew to the tallest lengths, as that is it's natural length, while Col-0 was shown to be most significant.
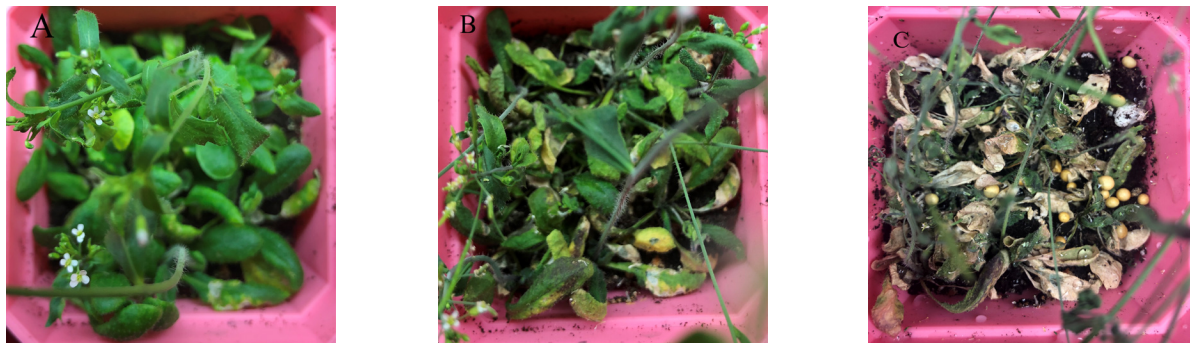
Figure 4: Ws-2 over a course of time. A) Ws-2 on day 24, after the 3rd Nickel Sulfate treatment. The plants grew very tall at a quick pace compared to other strains, however not significantly to control. B) Ws-2 on day 28, after 5th Nickel Sulfate treatment. The roots are beginning to turn yellow, wilt and die. This is most apparent in Ws-2 strains, compared to other strains. C) Ws-2 on day 35, end of experimentation. The leaves have wilted and died.

Chi Squared test had shown that there is no significant effect of metal on the Ws-2 strain, the p result = 1.7 (<5.99). Ws-2 generally grows to 200-250 millimeters. Day 29, the only significance statistically found from the T Test was the effect of copper sulfate on Ws-2, the p result = 0.02 (<0.05). On Day 35, Ws-2, copper sulfate (153.4 mm), nickel (142 mm) and chromium (167.7 mm) in comparison to the control (157.3) grew at a similar rate. The p result for Ws-2, copper = 0.419 (>0.05), showing no significant effect.
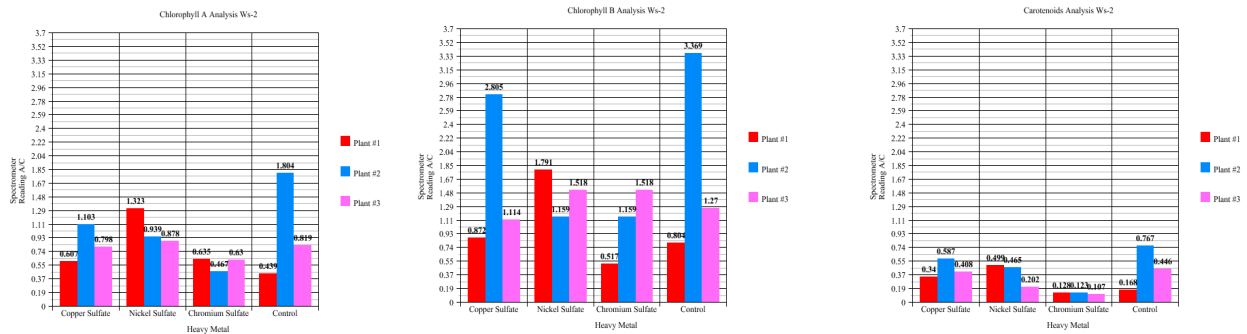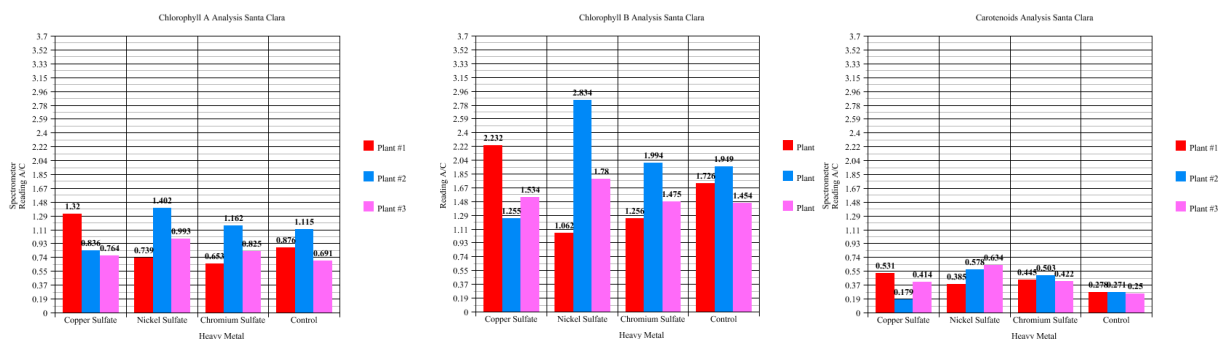

Graph 5: Chlorophyll A and B and Carotenoid levels in Ws-2 strain. Chlorophyll A Graph showed no distinctive results between the control and experimentals. Chlorophyll B graph shows a high concentration of chlorophyll B in copper sulfate and control plants. Carotenoid analysis showed no significant differences to consider between the control and experimental.

No significant effect was shown in the T Test for chlorophyll A, B and carotenoids of the Ws-2 strain experimentals and control.

3.4 Santa Clara Height and Health

The Santa Clara genotype had shown some significant difference in effect of heavy metals in comparison to the control. The Chi Square test gave a p result = 87.9 (>5.99), there is a significant effect present in the Santa Clara strain. However, a Student T Test had shown no significant effect (<0.05) in any of the Santa Clara plants exposed to nickel, chromium and copper sulfate on Day 29 and Day 35.

Graph 6: Chlorophyll A and B and Carotenoid levels in Santa Clara strain. Chlorophyll A Graph showed no distinctive results between the control and experimentals. Chlorophyll B graph shows that in Plant 2 the chlorophyll B concentration is higher than any other plants. Carotenoid analysis showed no significant differences to consider between the control and experimental

Another T test was conducted on the spectrophotometry results, and the carotenoids of Santa Clara plants exposed to nickel ($0.042 < 0.05$) and chromium ($0.006 < 0.05$) showed a significant effect. In comparison to the control (0.266 A) , carotenoids of nickel plants $= 0.532$ A and chromium $= 0.456$ A are increasingly larger indicating the carotenoid levels in the heavy metal exposed plants are healthier. Heavy metal exposed Santa Clara (nickel and chromium) may be considered healthier, suggesting that these metals increased their carotenoid and allowed them to flourish. Even though carotenoids showed a positive result in significance level, chlorophyll A and B levels had shown no significance in the experimentals and control.

3.5 BLAST Analysis: Col-0 and Lov-1

Nucleotide BLAST analysis between Col-0 and Lov-1 showed 3 significant differences. In Chromosome 1, Range 3: 17354618 to 17354639, there was a 9% difference between Col-0 and Lov-1. The features of the string of nucleotides code for F-box and associated interaction domains-containing protein. This protein regulates the cell cycle of the plants, flower development and hormone signals. The protein assists in regulatory processes in *Arabidopsis thaliana* (Kuroda, H., Takahashi, N., Shimada, H., Seki, M., Shinozaki, K & Matsui, M., 2002). The difference in nucleotide strings between the strains which code for the protein which regulates growth and hormones of plants may be the reason Col-0 was more resistant to the heavy metals, as the protein may be upregulated in Col-0, leading to the resistance of heavy metals.

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Query  252     ACCCAACTTTCCCGTGTCGTTT  273
               |||||||||||| ||| ||||
Sbjct  17354618  ACCCAACTTTCCCTTGTAGTTT  17354639
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Figure 5: BLAST Difference of F-Box Protein. The difference in the strings of nucleotides shows a difference in the code instructions to build proteins which regulate growth and hormones.

There was another 9% difference found between the nucleotides in Chromosome 1 in Range 5: 26262481 to 26262502. The features which these nucleotide string codes for are 2016 bp at 5' side: Homeobox-leucine zipper protein family (HD Zip) 2. There are four classes of HD Zip proteins. Class one responds to abiotic stressors, such as water and light, abscisic acid and embryogenesis. Class two controls auxin signaling and shade avoidance. Class three leaf polarity and meristem function, while class four creates differential epidermal cells, trichome formation, and root development. Studies on Class one, which work on abiotic stressors have shown upregulation of ATHB12 and ATHB7 during a water limited situation (Soderman, E., Mattson, J., Svenson, M., Borkird, C, & Engstrom, P., 1994). In addition, ATHB6 protein is a regulator of Abscisic acid (ABA) signal pathway and interacts with protein AB1. ABA is a plant hormone which functions in plant developmental processes, such as seed and bud dormancy, as well as plant growth (Elhiti, M & Stasolla, C., 2009). The difference in nucleotide strings which code for the HD Zip proteins is a crucial aspect of plant development, health and resistance to heavy metals. This may account for the differences in heavy metal resistance during experimentation.

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Query  343     ATGTACAATTCGATGATATCGG  364
               |||||||||| ||||||| ||
Sbjct  26262502  ATGTACAATTCTATGATATAGG  26262481
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Figure 6: Difference in nucleotide string for HD Zip protein. The difference in nucleotides shows the difference constructs to build the HD Zip proteins, crucial for many aspects of plant life.

There was an 8% difference in the nucleotide strings found in chromosome 2 of Range 2: 18632135 to 18632157. This nucleotide string codes for the Plant-specific transcription factor YABBY family protein. YABBY protein family is involved in many essential processes of plants such as growth in addition to tolerance of abiotic stress (Zhang, S., Wang, L., Sun, X., Li, Y., Yao, J., Nocker, S & Wang, X., 2019). The proteins are known to help in the development of organs such as cotyledons, leaves, young flower buds, and flower organs. The protein may have been upregulated in Col-0, causing organs to grow stronger and prevent heavy metals from entering the plant (Watanabe, K & Okada, K., 2003). This difference in nucleotide strings which code for the YABBY proteins may be an aspect which led to a higher expression of YABBY in one strain, than another, which caused greater resistance to heavy metals.

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Query  221      AGCTCTTGAAAAACAAATTTCAGT  244
                |||||||||||| |||||| |||
Sbjct  18632135 AGCTCTTGAAAAA-AAATTTAAGT  18632157
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Figure 7: Difference in nucleotide string for YABBY protein family. The difference in strings of nucleotides of YABBY may lead to different responses to stressors between the strains as well as growth.

3.6 BLAST Analysis: Col-0 and Ws-2

The nucleotide BLAST between Col-0 and Ws-2 showed two significant differences. In Range 1: 12058202 to 12058226 of chromosome 1 there was an 8% difference in the nucleotide string which codes for beta-1,3-n-acetylglu-cosaminyltransferase radical fringe. Beta-1,3-n-acetylglucosaminyltransferase radical fringe functions in transfer of glycosyl groups and is located in the chloroplast. It is expressed during growth of plants (Moloney, D., Panin, V., Johnston, S., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K., Haltiwanger, R, & Vogt, T., 2000). This difference of nucleotide strings encoding this may be a cause for the distinction in resistance between the heavy metals.

```
Query  34       AACAGCTTCAAGGTGATG-ATTGAG  57
                |||||||||| ||||| ||||||
Sbjct  12058202 AACAGCTTCAACGTGATGAATTGAG  12058226
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Figure 8: Nucleotide strings of beta-1,3-n-acetylglucosaminyltransferase radical fringe. The difference in nucleotides for Beta-1,3-n-acetylglucosaminyltransferase radical fringe, may account for the difference in resistance to heavy metals.

In Range 1: 4215061 to 4215082 of chromosome 3, there was a 4% difference in the nucleotide string which codes for the serine acetyltransferase 2 (SAT) gene family. Previous research has found that several genes of SAT increased in expression under heavy metal exposure of Cadmium in *Arabidopsis thaliana*. SAT genes response to Cadmium was found to be an increase in the rate of sulphate assimilation to tolerate the stress (Howarth, J., Solis, J., Alcala, G., Wray, J., Romero, L, & Gotor, C., 2002).The difference in nucleotide strings which code for the SAT gene family which increase expression during heavy metal stress, may be a cause to the difference in resistance during experimentation.

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Query  349      GACGTGCACGCCGCGTACGACGG  371
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Sbjct 4215082 GACGTGC-CGCCGCGTACGACGG 4215061

Figure 9: Difference in nucleotide strings for SAT: The difference in strings may lead to a difference in expression leading to different amounts of resistance.

4. Conclusion and Future Work

Our study supports the experimental hypothesis that the treatment of heavy metals affected the Col-0 genotype in a positive or negative way because it is considered the wild strain. The Col-0 strain exposed to nickel sulfate grew a substantial amount in comparison to the control, indicating the strain's resistance to this certain metal. However, the chlorophyll B concentration level in the Col-0, nickel sulfate plants were lower than the control, implying that the plants health was affected by the metal in a negative way. It makes sense that Col-0 was more resistant as similar results of Col-0 were found by other researchers who studied Col-0 under cold temperatures. Such research found Col-0 to be resistant to freezing temperatures, identified by gene ICE2 (Fursuva,O., Pogorelko, G, & Tarasov,V., 2008). Furthermore, a mutant of strain Col-0, ATR7, was found to regulate oxidative stress. AT5G59390, AT1G30170, AT1G21520 (proteins, DREB19, HSFA2, ZAT10 (factors of transcription) and CHR34 (remodel of chromatin) were found to elevate under ATR7 (Sujeeth, N., Mehterov, N, Gupta S., Qureshi, M., Fischer, A., Proost, S., et al, 2019). In addition, Col-0 has been previously found to contain the Ferric Reduction Oxidase gene family (FRO). The FRO gene family has recently been found to express under stressors (Mukherjee, I., Campbell, P., Ash, J, & Conolly, E., 2005). The rise in expression was examined under environmental stressors such as drought, heat, salt, heavy metals, etc. This depicts the importance of FRO in developement and stress (Muhammad, I., Jing, X., Shalmani, A., Ali, M., Yi, S., Gan, P., Li, W., Liu, W, & Chen, K., 2018). This wild strain of Col-0 has found a way to sustain multiple environments has found ways of resisting stressors and stressful environments such as cold environments, oxidative stress, certain heavy metals etc. This study has added to such claims, showing that compared to various other strains of Arabidopsis thaliana, Col-0 was more resistant to Copper, Nickel and Chromium sulfates.

BLAST analysis showed significant differences between different strains of *Arabidopsis thaliana* which explain the difference in resistance to heavy metals. Differences in the nucleotide strings which encode several genes and proteins may be responsible for the different responses observed during experimentation. Such differences in nucleotide strings included F-Box gene family, HD Zip, YABBY protein family, beta-1,3-n-acetylglucosaminyltransferase radical fringe and SAT. The BLAST showed differences in nucleotide strings which, in addition, explain reasons for increased amounts of resistance in strain Col-0. Based on the BLAST analysis, Col-0 is more resistant to heavy metals compared to other strains.

Future work would include the finding of the complete Santa Clara sequence, in order to conduct a BLAST between Santa Clara and Col-0. With the ability of having all four genotype sequences will allow the finding of one specific protein that caused Col-0 to be the most resistance in comparison to the other genotypes. This will allow for future genetic modification of the specific protein for other plants, allowing them to become resistant to heavy metals in the environment. Subsequently, such plants with the genetic modification will grow better in a stressed environment and will not pass toxic heavy metals through the food chain. The genetic modifications will make it safer for animals and humans to ingest such plants. Plants under genetic modification will have the stress resistant attributes of Col-0 due to the modification of certain stress resistant genes and proteins. This will make the crops eaten safer to consume as the toxic chemicals of heavy metals would not be passed through the food chain.

Acknowledgments

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