Identification of Organometallic Complexes in Agromining Extract

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Debora Lopes

Debora Lopes

Gisele Trivino

Gisele Trivino

Date:

Approved:

Stephen J. Kmiotek

Professor Stephen Kmiotek, Advisor

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Abstract

The goal of the project was to identify organic compounds within the agromining solution of non-incinerated Alyssum *murale* and Leptoplax *emarginata* biomasses, and assess their affinity to complex with nickel as they may be inhibiting nickel recovery. Through the use of fluorescence spectroscopy, the team identified the presence of the compound indole, which is likely attached to glucosinolate. In addition, the team detected the presence of two other organic compounds but an identification of the organic compounds was not made. The small sample size prevented our team from drawing any correlations between the organic compounds and their ability to complex with nickel. Further testing is needed in order to determine if there are any correlations between the identified compounds and nickel recovery.

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1. Introduction

Nickel is one of the most versatile and useful metals in the world with industrial, consumer, military, and even transport applications of this element (Minerals Make Life, 2015). Nickel's properties allow it to be used in a variety of applications thus the dependability of the supply of this heavy metal is crucial. However, as years progress, the nickel production from traditional mines has diminished due to the closing of many mines due to environmental and health implications (NIKKEI, 2016). As mines shutdown, the supply of nickel ore diminishes, but the demand for nickel keeps rising due to the demand of stainless steel, one of the main products produced from nickel (NickelInstitute, 2017). To keep up with the demand and to prevent further harm caused by the environmental and health implications of nickel, alternative methods of nickel production should be researched and looked into to supplement the current nickel stock. One of these potential alternates would be agromining.

Agromining is typically a combined process of agronomic, pyro- and hydrometallurgical procedures (Zang et al., 2014). A hyperaccumulator plant is used to extract nickel from the soil, which then becomes a part of the plants biomass. The plant is then harvested and the pyro- and hydrometallurgical procedures remove the nickel from the plant's organic matter. The nickel collected is a free metal, which then can be sold or reused in industry. This process is environmentally friendly because it removes the need for machinery to remove nickel from mines. However, this process may still cause nickel pollution due to the volatility of nickel and improper handling during the pyrometallurgical procedure.

The Sols & Eux laboratory is currently researching a primarily hydrometallurgical process to remove the nickel from the hyperaccumulator plant. This would decrease the potential polluting of the atmosphere via agromining and would establish a cheaper method of nickel extraction. This method would consist of using deionized water to extract a solution consisting of organic material and nickel from the plant biomass. Then, another procedure to remove the nickel from the organic material it complexes with to produce a free nickel similar to those produced from other agromining methods.

2. Background

Heavy metal soil contamination is a growing problem that many regions of the world, both industrial and rural, must face. Nickel, one of these metals, has been observed to have a detrimental impact on human and environmental health, but when extracted from the earth can produce significant economic and environmental benefits. Traditional nickel removal methods for remedial purposes are costly if the contaminated soil spans over a large area. In recent years, agromining has been studied as a possible solution due to the low cost and high yield of nickel in hyperaccumulators.

2.1. Nickel in Soil

In many cases, the presence of nickel in high concentrations can be attributed to nearby anthropogenic activities. In industrial regions, nickel contamination can be a result of improper tailing waste removal from mining operations, metal plating factories, power plants or waste incinerators emissions (Wuana & Okieimen, 2011). If introduced into the environment through smokestack emissions, the nickel will settle into the ground and accumulate near the top of the soil with a uniform dispersal throughout the soil profile. Wuana and Okieimen studied the effects of this form of pollution and concluded that nickel emitted into the atmosphere via anthropogenic activities pose a greater threat to humans and the environment than naturally occurring nickel. This is because the nickel emission rate is "one-to-three times greater than natural fluxes" and is more bio-available. Once introduced into the environment, stabilization of the metal may be difficult as the local fauna may not be accustomed to a high concentration of a toxic metal. The effects of nickel on the environment will be discussed further in *Health and Environmental Implications of Nickel.*

Nickel pollution is not solely an industrial area concern; nickel soil contamination has become a growing problem in farming and agricultural areas. Nickel is primarily introduced to the soil directly through the use of traditional fertilizers and sewage sludge (Wuana & Okieimen, 2011). In the United Kingdom, farms that use these methods to increase their crop yield have observed nickel concentrations in soil at 385 mg/kg while the recorded background concentration is 25 mg/kg (Li et al., 2003).

Although nickel is typically known to be a soil contaminant caused by anthropogenic activities, the heavy metal is also known to be naturally occurring in serpentine soil (USDA Forest Service, n.d.). Serpentine soil can be found in many areas of the world but is most common in the mountain ranges of the United States and the Balkan region of Europe (Shallari et al., 1998). The ultramafic serpentine soil is characterized by a high concentration of heavy metals – including nickel, cobalt, and chromium – high pH, and low concentration of macronutrients (Shallari et al., 1998). The nickel concentration within the soil tends to be several g/kg of soil but has been observed to exceed a concentration of 10 g/kg. Due to the soils distinctive composition, the plant life inhabiting these areas have developed unique mechanisms that allow them to uptake heavy metals without signs of heavy metal toxicity. Many species in these areas are considered to be endemic to the region and have been labeled as hyperaccumulators (Zang et al., 2014).

Soil contamination of any kind is a concern as it provides a pathway of exposure of toxic materials

to humans and the environment. Nickel and other heavy metal contaminants are especially worrisome as soil is a large sink for metals. Once in the soil profile, nickel will not undergo biological decomposition like organic compounds or nutrients would (Wuana & Okieimen, 2011). It will remain in the system until stabilized by a plant, which may then die due to exposure and heavy metal toxicity. In addition, the accumulation of nickel reduces the pH of soil, enabling the mobility of nickel and with time may contribute to acid rain that can affect large areas of land. Nickel concentrations varies worldwide and range from 0.2 – 450 mg/kg of soil with a mean of 22 mg/kg (Iyaka, 2011).

2.2. Health and Environmental Implications of Nickel

The importance of nickel removal from soil can be attributed to nickel's effect on the quality of human and animal health, its environmental impact, and its economic significance around the world.

Nickel is a naturally occurring metal in the ground, and it is essential element for plant life (Buechel, 2016). However, the presence of excessive nickel in the soil is harmful, not only to the fauna, but to the humans and animals that consume the vegetation. Animals and humans that cannot properly digest nickel are being exposed to it through ingestion of food and through direct contact, such as in the workplace.

The United States' Occupational Safety and Health Administration (OSHA) and Environmental Protection Agency (EPA) have both set enforceable exposure limits for nickel in the workplace and overall. The recommended permissible exposure limits (PELs), established by OSHA, for nickel metal, water-soluble and water-insoluble nickel compounds are 1.0 mg Ni/m^3 (NickelInstitute, 2008). The EPA recommends that nickel presence in water should not exceed 0.1 mg/L (ATSDR, 2015). In the section *Nickel in Soil*, the concentration of nickel in soil sources are discussed. Those nickel levels are within the limits set by governmental bodies; however, constant exposure can lead to serious health issues in humans and animals.

In humans, exposure to nickel can lead to liver, kidney, spleen, and brain damage; and may lead to vesicular eczema, lung or nasal cancer. In animals, exposure to nickel can lead to tissue damage, reduced reproduction and development, or death (Scott-Fordsmand, 1997).

Nickel's most harmful property is that it is a carcinogen; the presence of nickel increases the chances of breast and gastrointestinal cancer in humans. Studies have shown that nickel acts like a pseudo-estrogen by binding to estrogen receptors and causing cell growth (Aquino et. al, 2012 & Mulware, 2013). Multiple studies have revealed that a lifetime overexposure to estrogen leads to breast cancer. Another study performed in the Van Region in Turkey indicated that the consumption of food crop harvested in soils with excessive amounts of heavy metals, including nickel, could be the cause or a factor of the endemic gastrointestinal cancer affecting the region (Kürsad Türkdoğan, 2002).

Nickel is one of the key metals that helped lay the foundation for the modern industrial world (Minerals Make Life, 2015), yet the traditional mining of this metal augments the greenhouse gases in the atmosphere and pollutes the land around it. In the section, *Nickel in Soil*, the reasons how

nickel pollutes the surrounding soil is discussed. The implications of this contamination adversely alter the surrounding habitat.

Traditional strip mining of nickel leads to increased concentration levels of nickel in that area by introducing the heavy metal to the surrounding land (Förstner, 1981). Nickel is an essential nutrient in plant growth, and nickel deficiency in plants can lead to delayed and stunted growth (Buechel, 2016). However, an over-consumption can also lead to similar issues. Thus, soils with elevated levels of nickel are detrimental to the health of the plant. This leads to the decline of fauna in the area.

A harmful side effect of traditional nickel mining is its contribution to global climate change by the introduction of more greenhouse gases in the atmosphere. Nickel mining requires the use of fossil fuels to operate, and as the demand of nickel increases, discussed in the section *Economic Importance of Nickel*, the production of nickel will increase to meet the demand. Thus, an increase in energy costs and greenhouse gas emissions are being generated from the mining of this heavy metal (Mudd, 2010).

2.3. Agromining: Fauna and Nickel Extraction

Agromining is a relatively new term and may also be referred to as phytomining. Simply, agromining is the combination of an agronomic process and a pyro- or hydrometallurgical process (Zang et al., 2014). During the agronomic process a hyperaccumulator is used to extract and stabilize nickel in soil. As the plant matures, the nickel will become a part of the plant's biomass. What distinguishes this style of nickel removal from others is the "mining" aspect. In agromining, the end goal is to be able to extract the nickel from the plant in order to reuse it. To do this pyro- or hydrometallurgical processes are implemented. Ashing, leaching, or a combination of the two are used to extract nickel or nickel products with the intention to be used in industry or sold. A key factor in ensuring a high nickel yield in the end product is the selection of the correct hyperaccumulator.

2.3.1. Hyperaccumulators

Alyssum *murale*, commonly known as Yellow Tuft, is an herbaceous perennial plant native to Southeast Europe (Missouri Botanical Garden, 2017). This plant is common in rocky, well-drained and non-habitable soils. The primary attribute of this plant that has led to further analysis is its hyperaccumulation of metals - specifically nickel (Oregon's Weed Watcher Program, 2017). Hyperaccumulating plants uptake over 1000 mg/kg of metal in dry matter through the soil they inhabit (Brooks et. al, 2001). The method that allows a plant to hyperaccumulate involves various processes in the plant; it involves "bioactivation in the rhizosphere, root absorption and compartmentation, xylem transport and distribution and sequestration," (Zhang, 2014). These hyperaccumulation processes can also be affected and/or dependent on the pH of the soil and fertilizers. These attributes allow Alyssum *murale* to amass a high quantity of nickel, which can be harvested through various treatments like ashing and leaching.

2.3.2. Agromining Techniques

There are currently three commonly used agromining methods that result in a high percent nickel yield. Two of the methods generate pure nickel by either a pyrometallurgical process or a combination of pyro- and hydrometallurgical processes. The third method also utilizes a pyro-hydrometallurgical process, but the end product is ammonium nickel sulfate hexahydrate (ANSH). Although these three techniques have proven to be feasible in a large-scale operation, in recent years there have been studies to establish a method to produce nickel or nickel products with a strictly hydrometallurgical process.

However, all methods begin with the same first step, the growth of a hyperaccumulator plant.

2.3.2.1. Agronomic Process

Ensuring a high nickel yield in the end product begins with providing optimum conditions for plant maturation and hyperaccumulation. A study conducted by Chaney et. al in 1998 revealed four soil parameters that will result in high heavy metal hyperaccumulation. These parameters are often followed when agromining.

- **pH:** Increasing the probability of nickel reaching the roots of the plant, or in other words improving the bioavailability of nickel, will in turn increase the amount the plant will uptake. To do this, the soil pH should be maintained in the 4.5 to 6.2 range since pH has a significant impact on the mobility of nickel (Chaney et al., 1998). The presence of nickel in the soil will naturally result in a soil with lower pH, but if not in the described range, the rate of nickel phytoextraction will be low.

- **Calcium Concentration:** A. *murale* has evolved mechanisms to thrive in serpentine soils, which are known to have low calcium concentrations. When sown into non-serpentine soil, calcium levels should be a maximum of 20% of the magnesium concentration in the soil. This calcium concentration may be obtained through acid leaching (Chaney et al., 1998).

- **Ammonium Fertilizers:** When agromining, healthy plant growth will aid in hyperaccumulation. Although A. murale is accustomed to grow in soil with heavy metals, the utilization of fertilizers with a substantial ammonium concentration will support the plant's health and growth (Chaney et al., 1998).

- **Chelating Agents:** The use of chelating agents will increase the bioavailability of nickel, similar to the effect of decreasing the soil pH. EDTA and NTA may be used to increase the bioavailability and selective uptake of nickel. If chelating agents are not used, A. murale will absorb magnesium, calcium or iron at a rate that inhibits the absorption of nickel. (Chaney et al., 1998).

When the A. *murale* has matured, it may be harvested by cutting the plant at the base. Due to the ability of hyperaccumulators to store nickel in aboveground tissue, there is no need to harvest the roots. The plants are then left to dry in the sun. The preparation for A. *mural* for nickel extraction entails grinding or crushing the dried plant. Prior to crushing, all moisture must be removed from the plant. The end result will be a powdery consistency when crushing steams and a flakey consistency when crushing leaves or seeds.

A variety of techniques may then be used to extract the nickel from crushed A. *mural.*

2.3.2.2. Extraction of Pure Nickel

Pure nickel may be produced two ways, one being a strictly pyrometallurgical procedure and the second being a pyro-hydrometallurgical combination. The former requires A. *mural* to be placed in an incinerator until it is reduced to ash. Incineration will burn any organic material. The ash is then placed in a smelter at 500-1500 ˚F to remove impurities and produce pure nickel (Chaney et al., 2007).

A combination of pyro-hydrometallurgical processes will result in a similar product but with a greater nickel recovery. In this method, similar steps are taken to produce A. *murale* ash. The ash is then leached with acid. The leachate will be highly concentrated in nickel, which can be extracted via electrowinning (Ent et al., 2015).

2.3.2.3. Extraction of Ammonium Nickel Sulfate Hexahydrate

In addition to producing pure nickel, pyro-hydrometallurgical processes are used in order to produce ammonium nickel sulfate hexahydrate ($Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$). This technique has been patented, and the ANSH crystals may be valuable to industry. A detailed flow diagram of the process may be seen in Figure 1.

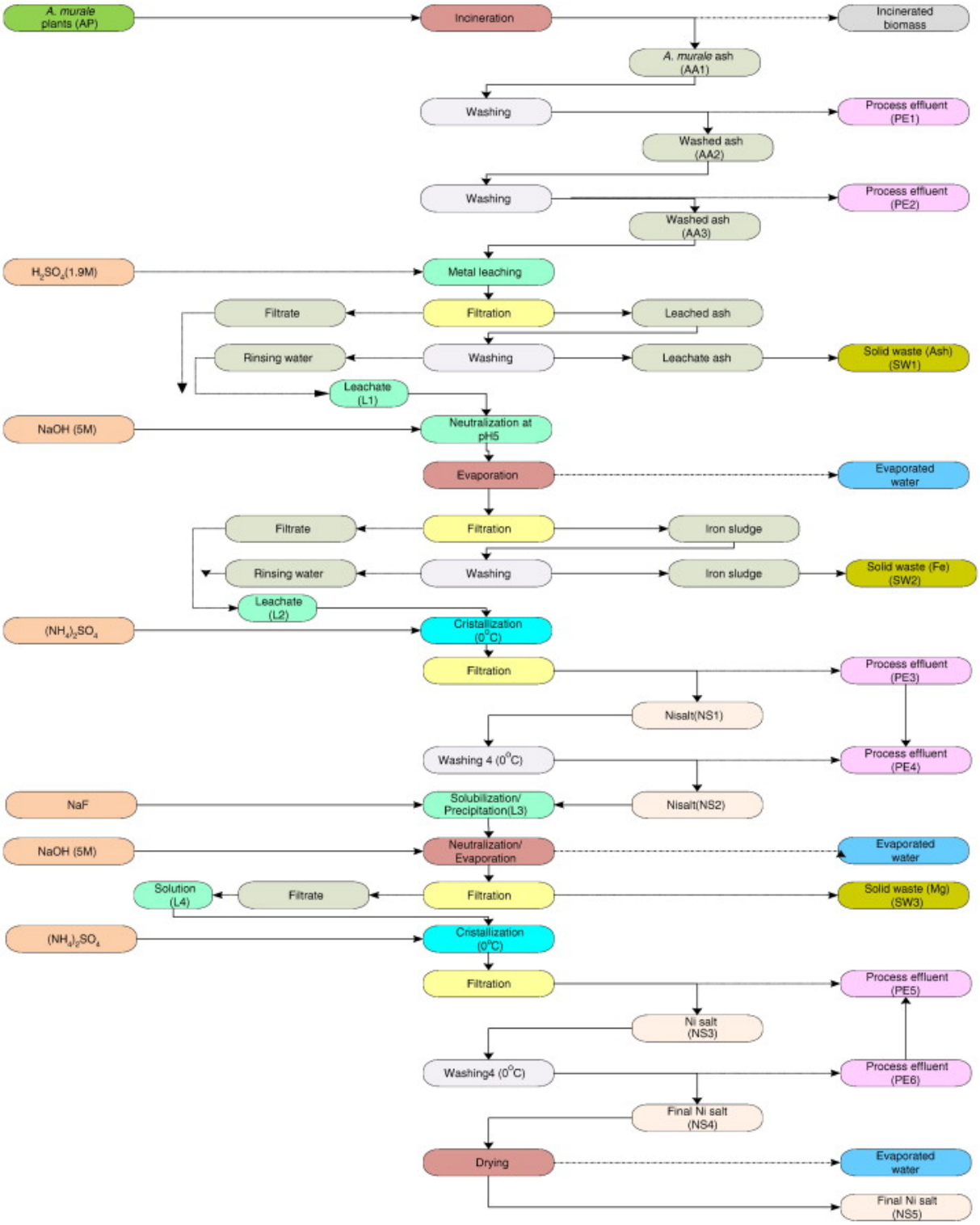

Figure 1: Ammonium Nickel Sulfate Hexahydrate (Barbaroux et al., 2012)

The process begins with washing the ashes A. *murale* twice in order to remove water-soluble potassium. The washed ashes are then leached with sulfuric acid to mobilize nickel, producing a leachate that has a high nickel concentration. Following a pH adjustment and partial evaporation,

the leachate is filtered using a magnetic filtration system. In order to produce crystals, $(NH_4)_2SO_4$ is added to the leachate stoichiometrically to the quantity of nickel. These crystals are then washed, and magnesium is precipitated out via a pH adjustment. Finally, the solution is filtered and allowed to crystallize, producing ANSH that is 13.2% nickel (Barbaroux et al., 2012).

2.3.2.4. Extraction of Nickel Cathode: A New Method

In recent years, research has been conducted in order to develop a method to extract nickel from A. *murale* using a strictly hydrometallurgical process. One reasoning behind this is that when A. *murale* is ashed, the exhaust may actually cause further nickel pollution if the nickel is volatilized and not properly captured.

In 2001, Barbaroux and his team ran a series of experiments to agromine nickel from A. *murale*, where leaching is the primary method for separation of nickel from the plant biomass. In this process, only the seeds of the plant were utilized due to their high nickel content as a result of the harvesting period.

To begin, the seeds were crushed, leached with sulfuric acid, and passed through a magnetic filtration system. This process resulted in 97% nickel transfer into the leachate solution. The residue on the filter was then washed twice with deionized water; this water was then combined with the leachate to produce a "global leachate". Several methods were tested to determine their capability of removing nickel from the global leachate solution.

Barboux and his team tested four nickel extraction methods: selective precipitation, electroplating, a coagulation-flocculation process, and solvent extraction. Of these methods, selective precipitation, electroplating, and coagulation-flocculation were all deemed ineffective due to the presence of organic material. When organic material is present in the solution, it will complex with a large portion of nickel and not allow for nickel extraction unless the bond is broken. This complication is a result of not incinerating the plant material. The final method tested was solvent extraction with a Cyanex 272 solution paired with electroplating. This resulted in approximately 72.4% nickel cathode recovery. In this experiment, the nickel-organic complexes were broken, which resulted in free nickel that was recovered via the electroplating process. Although, this method did result in high nickel recovery, an economic analysis conducted came to the conclusion that this process was economically unfeasible on a larger scale (Barbaroux et al., 2012).

2.3.3. Economic Importance of Agromining of Nickel

Nickel is one of the most versatile heavy metals used in everyday to industrial applications. The properties that make nickel a sought after metal are it's: corrosion resistance, strength at a variety of temperatures, and toughness (NickelInstitute, 2017). From the International Council of Mining & Metals (ICMM), "Today's staples of iron ore, copper, gold and nickel, will remain the most important investment targets for mining companies," (Ericsson et. al, 2012). Nickel will remain an essential metal to be mined, and it will continue to be utilized by various companies, thus the development of new techniques or technologies to harvest this metal are necessary to curb its environmental and health implications.

The demand for nickel has increased due to it being a principal raw material in the production of stainless steel. There are a variety of types of stainless steel, but on average, stainless steel contains approximately 8-10% nickel (AZOM, 2013). Due to the manufacturing of stainless steel requiring nickel, the stainless steel industry accounts for over 60% of nickel sales (NickelInstitute, 2017). Countries around the world, primarily countries with strong industrial sectors, are demanding more stainless steel as they continue to build. However, nickel inventories around the world are decreasing worldwide (NIKKEI, 2016). The supply shortage of nickel is due to many mines closing down because of health concerns (Listiyorini, 2016). Also, the nickel ore supply has been vastly depleted from mines.

A solution to boost nickel supply would be to agromine the metal. Not only does this technique increase the supply of this metal in the world, but also it combats the environmental and health issues associated with the production of nickel. The main contributions of agromining would be the rehabilitation and repurposing of the land, which provides another source of nickel and boosts the European economy.

This viable method would add another source for nickel collection, which would allow many stainless steel industries to keep expanding. By generating nickel from a non-traditional mining source, it would supplement the nickel generated from traditional mines and allow the supply to meet the demands.

As discussed in *Nickel in Soil* and *Health and Environmental Implications of Nickel,* the presence of nickel in soil is an environmental hazard. Currently, the United States and many other countries are attempting to cleanup contaminated soils. The current method of rehabilitation of heavy metal contaminated land is to excavate the area and bury the soil in a hazardous waste site. However, using the conventional method to cleanup soils with heavy metals is costly. The United States has invested an estimated USD$300 Billion (Raskin, 1997), and it costs approximately USD$1,000,000 per acre to cleanup a site. Not only are these methods expensive, but they only remove the soil and do not attempt to treat it.

Agromining treats the problem of soil contamination by repurposing and rehabilitating the soil. The method takes previously unusable land and with the use of a hyperaccumulating plant, removes the nickel from the soil. After the plants are harvested, the area can then be used as farming lands due to the removal of the toxic metal. The harvested plants are then processed to recover the nickel. This would not only revitalize the area, but also boost the economy (van der Enta et. al, 2013).

The European Union (EU) identifies nickel as a “raw material with high economic importance,” (Nodot, 2016). However, most of the nickel production occurs outside of the EU resulting in a loss of potential income for the EU. In the EU, there are thousands of acres of ultramafic soils that are viable for nickel extraction via agromining (Nodot, 2016). Currently, nickel accounts for €80-100 billion in the European economy “of which around €50 billion is estimated to be generated by industries and applications that are critically dependent on nickel,” (NickelInstitute, 2017). The presence of nickel enables 1.25-1.50 million jobs being employed in the EU (NickelInstitute, 2017). With the introduction of agromining in the EU, domestically harvested nickel could potentially add revenue and jobs.

Agromining is continuously being researched especially as there is still no solidified method to extract nickel via a strictly hydrometallurgical process. There are many complications with this process due to the presence of organic material and an inability to uncomplex nickel from the organics. Our research will focus on identifying the organic complexes that may prevent the collection of nickel.

3. Methodology

In this section, the methods of preparing the plants for nickel and organic material extraction and the analysis of the extracted solution are explained. The procedure for each process is described in their respective section. These procedures are divided into two phases: sample preparation and sample analysis. The order of these procedures is listed in their respective sections, but the procedures for sample preparation must precede sample analysis. In addition, this section will describe the cultivation characteristics of the tested plants. During all procedures, the proper personal protective equipment (PPE) was used in the laboratory, which includes, but is not limited to: safety glasses, lab coats, disposable gloves, and facemasks.

3.1. Plant Cultivation

Two species of hyperaccumulators were tested throughout the experimental time period, Leptoplax *emarginata* and Alyssum *murale*. In addition to testing different species of hyperaccumulators, each species was cultivated in two distinctive manners, which resulted in there being four plant samples.

3.1.1. Leptoplax *emarginata*

Two samples of Leptoplax *emarginata* were tested, each was cultivated in the same manner on serpentine soil located in Spain. Each sample was grown on identical plots, with similar soil profiles and nickel content. These two samples will be referred to as Leptoplax 2 (L2) and Leptoplax 3 (L3).

3.1.2. Alyssum *murale*

Two methods were implemented for the cultivation of Alyssum *murale*. The samples the team analyzed were grown in serpentine soil plots located in Spain. As previously described, serpentine soil is characteristically low in nitrogen, which can inhibit plant growth. To mitigate this problem, one sample was grown simultaneously with legumes, which are known to fix nitrogen. This sample is referred to as Co-Culture (CC). The second Alyssum *murale* sample was also grown in Spain on serpentine soil, but fertilizers were used in place of legumes. This sample is referred to as Co-Culture Plus (CC+).

3.2. Sample Preparation

The sample preparation phase is comprised of two procedures: *Plant Preparation* and *Extraction of Organic Material and Nickel with Water.* These procedures allow for the separation of different plant components to be made into extracted solution, which will be analyzed utilizing the procedures in the *Sample Analysis* phase. This phase must be done in chronological order with plant preparation first then the extraction procedure occurring.

3.2.1. Plant Preparation

1. Acquire one plant that will be tested, whether it is Alyssum *murale* or Leptoplax *emarginata*. One plant is determined to be a series of stems that extend from a singular root, as pictured below in Figure 2.
2. Separate the stems, leaves, flowers (if there are any), and seeds (if there are any) from each other.
3. Tare a dish and mass the separated stems, leaves, flowers, and seeds.
4. Insert the dishes into the autoclave to dry. It must be set to 100^{o}C and allow the materials to dry overnight to remove moisture.
5. Remove the dishes from the autoclave and let it cool in the dehydrator until it is cool to the touch.
6. Mass the dishes of plant material.
7. Using a miniature blender under a ventilation hood, grind the plant materials to a fine dust.
8. Mass the pulverized material and then insert into a container. This material will be used in the *Extraction of Organic Material* procedure.

Figure 2: A plant sample stemming from a singular root

3.2.2. Extraction of Organic Material and Nickel with Water

1. Using the material from the *Plant Preparation* procedure, mix 96 mL of deionized water with 4 grams of plant material. If 4 grams of plant material is not present, utilize the equation below to determine the appropriate amount of deionized water to add.

$$mL\ of\ Water = \frac{(1 - 0.04) * Mass\ of\ Plant\ Material\ (g)}{0.04}$$

2. Add a stir bar to the beaker that contains the mixture and let it mix for 30 minutes at medium speed (500 rpm).
3. After mixing, remove the magnetic stir bar. Acquire a vacuum filtration system and use it to separate the liquid solution from the solid plant biomass.
4. Measure the volume of solution, and then add 35 mL to a test tube. The rest can be dispensed. If less than 35 mL is present, then add all of the volume to the test tube.
5. Place the test tube in the freezer to be used later on in the week for analysis or in the refrigerator if it will be utilized later on in the day.
6. Mass the plant material and then insert it to the autoclave to be dried. Allow the plant biomass to dry overnight, and then mass the biomass for a final weight.

3.2.3. Storage of Agromining Extract

The plant biomass utilized in *Extraction of Organic Material and Nickel with Water* may be stored at room temperature in a sealed container. However, the extracted solutions must be stored in the freezer or refrigerator to prevent degradation of the organic matter. Once the *Sample Analysis* phase has started, allow the frozen samples to thaw in a bath of warm water. Once thawed, use the samples that day. If those samples are needed for the following days, store the materials in the refrigerator. Samples that have undergone *Mineralization* may be stored at room temperature as the process removes any trace of organic material.

3.3. Sample Analysis

The sample analysis phase resulted in three separate outcomes: the Characterization and Identification of Organic Compounds in Solution, Determination of Metal Concentrations in Solution, and Determination of Nitrogen and Organic Carbon Concentrations in Solution. These conclusions were the end result of several separate analysis techniques: UV-Visible Spectroscopy, Fluorescence Spectroscopy Analysis, Mineralization, Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) Analysis, Nitrogen Analysis and Total Carbon (TC) Analysis. These techniques contributed to their respective end results, which will be detailed later in this section. UV-Visible Spectroscopy testing must be performed prior to Fluorescence Spectroscopy Analysis and Mineralization must precede ICP Analysis as well. All other procedures in this phase can be completed simultaneously and do not need to occur one after the other. They may also be completed on separate days.

3.3.1. Characterization and Identification of Organic Compounds in Solution

To proceed with developing a method to recover nickel from hyperaccumulators, the organometallic complexes that are present in the agromining solution produced from *Extraction of Organic Material and Nickel with Water* must be identified. Previous studies on dissolved organic matter in water bodies have shown that this may be accomplished by utilizing fluorescence spectroscopy (Ohno, 2002; Huguet et al., 2009; Mcknight et al., 2001). Fluorescence indexes will

also be calculated in order to characterize the dissolved organic matter based on age and aromatic complexity.

3.3.1.1. UV-Visible Spectroscopy

An Anthélie Light Spectrophotometer and 1 cm Hellma quartz cuvette were used in order to obtain UV-visible spectra for each plant extract sample obtained from *Extraction of Organic Material and Nickel with Water*. The samples were diluted by a factor of 100 and the tested wavelengths ranged from 200-600 nm. The primary purpose of UV-visible spectroscopy is to determine whether the sample needs further dilution, as the inner-filter effect, which will be discussed later in this section, will impact the fluorescence results. Absorbance intensity results less than or equal to 1 cm^{-1} were desired during testing as the inner-filter effect would be negligible. If the absorbance intensity was approximately 3.5 cm^{-1} or less, the fluorescence results would need correction for the inner-filter effect but further dilution would not be necessary.

3.3.1.2. Fluorescence Spectroscopy Analysis

Fluorescence spectra were obtained for all samples using a Hitachi F-2500 Fluorescence Spectrophotometer equipped with a xenon lamp and a plastic disposable 1 cm cuvette. The *Extraction of Organic Material and Nickel with Water* samples were diluted to a factor of 100, which was deemed adequate through UV-visible spectroscopy. In order to analyze the data, the organic compounds present in the samples were identified and three indexes were used to characterize the hyperaccumulator extracts: Humification Index (HIX), Biological Index (BIX), and Fluorescence Index (FI). The characterization methods the team implemented required fluorescence spectra to be obtained with excitation wavelengths (λ_{exc}) of 254 nm, 310 nm, 370 nm, and one where the emission wavelength (λ_{em}) was 50 nm greater than the excitation wavelength. A detailed description of these detection methods will be described later in this section.

Humification Index (HIX)

The humification index has been used in past studies to analyze the degree of humification of dissolved organic matter (DOM) (Ohno, 2002). As an organic compound becomes more humified, lower weight organic compounds are converted to higher molecular weight compounds. The implications of this index will be discussed further in the **Results & Discussion** section. The degree of humification may be calculated using a modified version of Zsolnay's HIX equation described below with λ_{exc}=254nm:

$$HIX = \frac{\sum I_{435-280}}{\sum I_{300-345} + \sum I_{435-480}}$$

Results will be on a 0-1 scale with 0 indicating a non-humified substance. The intensities were corrected by subtracting the spectra of deionized water for the corresponding wavelength.

Biological Index (BIX)

In previous studies of dissolved organic matter, the presence of fluorophore beta has been used to assess the relative age of the DOM, as its presence is indicative of recent autochthonous biological

activity (Huguet et al., 2009). The implications of this index will be discussed further in the **Results & Discussion** section. The biological index may be calculated using the following equation with a λ_{exc}=310 nm.

$$BIX = \frac{I_{380}}{I_{430}}$$

Results will be on a 0-1 scale, 1 indicating recently autochthonous activity. The intensities were corrected by subtracting the spectra of deionized water for the corresponding wavelength.

Fluorescence Index (FI)

The fluorescence index is commonly used to analyze humic substances in natural bodies of water. Although the samples the team prepared are plant based, the fluorescence index may still be used in order to differentiate the organic matter in one species or plant component from the other. The implications of this index will be discussed further in the **Results & Discussion** section. To calculate the fluorescence index, the sample should be excited with a wavelength of 370 nm and the following equation proposed by McKnight and team (2001) used:

$$FI = \frac{I_{380}}{I_{500}}$$

Results of the FI index typically range from 1-2, but they may also fall outside of this range.

Identification of Organic Compounds

To identify the organic compounds present in the agromine solution, a synchronous-50 fluorescence absorbance test was run on all samples. The excitation wavelength ranged from 230 nm to 600 nm, with the emission wavelength being detected at 50 nm greater than the excitation. The fluorescence results of the test were corrected for the inner-filter effect which occurs when a sample has a high absorbance of the excitation and emission wavelengths used in a fluorescence test, causing the recorded intensity to be skewed (Mcknight et al., 2001). For this reason, it was necessary to run a UV-visible spectroscopy test for all samples to determine whether fluorescence results needed corrections via an equation or whether the sample must be diluted further. Fluorescence intensities of samples with an absorbance of 3.5 cm^{-1} or less were corrected with the following equation:

$$I_{corr} = I_{detect} * log\left[\frac{Abs_{exc} + Abs_{em}}{2}\right]$$

In the equation, I_{corr} is the corrected fluorescence intensity, I_{detect} is the fluorescence intensity detected by the spectrophotometer, Abs_{exc} is the absorbance of the excitation wavelength determined from the absorbance test and Abs_{em} is the absorbance of the emission wavelength (50 nm greater than the λ_{exc}).

The corrected results were then run through a dissolution program developed by a doctor in the

Sols & Eux laboratory. This program analyzes the fluorescence results to determine the number of fluorophores present in a solution.

3.3.2. Determination of Metal Concentrations in Solution

Mineralization

To measure the metal concentrations in the agromine samples, the organic material must be destroyed prior to *ICP-AES* testing through the process of mineralization. The presence of organic material will interfere with the testing, as the organometallic complexes within the solution will not allow for a reliable reading. A Milestone Smart D Microwave Digestion machine was used in combination with nitric acid to complete the mineralization process. After, the organic matter is destroyed; the solution is then analyzed via ICP-AES, which is described below.

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) Analysis

A PhD student in the Sols & Eux laboratory performed the ICP-AES analysis. The results will be discussed in the **Results & Discussion** section of the paper. However, it is noted that the team prepared the samples for the ICP-AES analysis. Approximately, 8 mL of the solutions from *Extraction of Organic Material and Nickel with Water* were added to 25 mL glass bottles via a Phenex RC 0.45 μm filter. Via ICP-AES, the concentrations of various heavy metals can be observed.

3.3.3. Determination of Nitrogen and Organic Carbon Concentrations in Solution

A professor in the Sols & Eux laboratory using a Shimadzu Total Organic Carbon Analyzer performed the Total Carbon analysis. The results will be discussed in the **Results & Discussion** section of the paper. However, it is noted that the dilution samples for the TC analysis was prepared by the team. The solutions from *Extraction of Organic Material and Nickel with Water* were diluted by a factor of 10 for the first experiment and then diluted by a factor of 50 for the second experiment.

4. Results and Discussion

In this section, the team will analyze the results obtained from the **Methodology** and discuss their implications. The results will be organized by the methodology phase, which produced them: *Sample Preparation* and *Sample Analysis.* Over the course of our experiments, two distinct trials were run. The first involved carrying out all procedures with two plants for each hyperaccumulator sample (L2, L3, CC, and CC+) and separating each by plant component (stem and leaf), resulting in sixteen distinct samples. During the second trial period, one plant from each hyperaccumulator sample was tested, but they were not separated by plant component. In addition, during the second trial period a second CC+ plant was tested and separated by plant component, resulting in a total of five samples for the second trial period.

4.1. Sample Preparation

For all trials, the samples were ground to produce a fine dust material that would be combined with water which would be vacuum filtrated. During these processes, the team encountered repeating issues that will be discussed in this section to prevent repetition in the sections below.

When the team ground the plant biomass, the material loss values were kept relatively low to reduce the amount of wasted material and to ensure the maximum potential extraction of nickel from the plant sample. However, it was difficult to retain all the material and prevent loss due to the nature of the fine dust. Also, it was observed that post-grinding the material would become static and adhere to metal objects. It was difficult to remove all the ground material from the blender thus not all the material could be recovered. This difficultly resulted in samples to have some material loss occur.

Vacuum filtration occurred to extract liquid solution from the biomass and water mixture. While performing the vacuum filtration, there were several instances where the procedure would be hampered by the biomass clogging the pore space in the filter paper. During these events, the biomass that remained still retained moisture; however, the filtration would not remove the liquid even after attempts were made to remedy the problem. Thus, this caused an inconsistency in the runs.

4.1.1. Leptoplax *emarginata* Samples

Following the procedures for sample preparation, Leptoplax 2 (L2) and Leptoplax 3 (L3) samples were prepared during the week of January 17th. Two plants from the L2 sample selection and two plants from the L3 selection were gathered. The leaves, stems, flowers (if present), and seeds (if present) of each plant were separated. The plant components were ground and the loss of material was measured. In Appendix A.1, the raw data for the collection portion of the procedure can be seen, and in Appendix A.2, the raw data for the grinding portion of the procedure can be seen. The average loss of plant material during this step was 6.55%. The material loss values for each plant sample can be seen in the table below.

Table 1: Material Loss for Leptoplax Samples

Sample	Material Loss Percentage
1 - L2 Stem	3.26%
1 - L2 Leaf	1.93%
2 - L2 Stem	0.73%
2 - L2 Leaf	2.41%
1 - L3 Stem	1.90%
1 - L3 Leaf	4.72%
2 - L3 Stem	1.15%
2 - L3 Leaf	36.26%

All Leptoplax samples contained flowers, but the mass of the flowers present was very minimal, which led to an inadequate quantity that could not be used for extraction. However, these flower samples were ground. Only the L3 samples contained seeds, and these seed samples were ground. However, an extraction of these seeds was not performed because a comparison could not be performed with L2. The seed and flower ground material were excluded from the average loss calculation.

Mass balances of each plant sample were conducted with their post-grinding mass. Below is a table of each sample's mass balance.

Table 2: Mass Balance for 1-L2

1 - L2			
	Mass (g)	**Total Mass (g)**	**Wt. Percentage of Plant**
Stem	25.82	30.4	84.93%
Leaf	3.9		12.83%
Flower	0.68		2.24%

Table 3: Mass Balance for 2-L2

2 - L2			
	Mass (g)	**Total Mass (g)**	**Wt. Percentage of Plant**
Stem	11.84	13.63	86.87%
Leaf	1.62		11.89%
Flower	0.17		1.25%

Table 4: Mass Balance for 1-L3

1 - L3			
	Mass (g)	**Total Mass (g)**	**Wt. Percentage of Plant**
Stem	33.54	39.22	85.52%
Leaf	2.02		5.15%
Flower	0.05		0.13%
Seed	3.61		9.20%

Table 5: Mass Balance for 2-L3

2 - L3			
	Mass (g)	**Total Mass (g)**	**Wt. Percentage of Plant**
Stem	41.11	44.13	93.16%
Leaf	0.58		1.31%
Flower	0		0.00%
Seed	2.44		5.53%

Based on the calculations, the plant's biomass is mostly comprised of stem material with an average of 85.90wt% for L2 and 89.34wt% for L3. The averages for leaf mass were 12.36wt% and 3.23wt% for L2 and L3, respectively. The masses of the specific plant components were used to normalize the final nickel concentration from each section of the plant. This normalization allowed the team to draw more definitive conclusions on what section of the plant provides a higher extracted concentration of nickel.

From the collected ground stem material, there was enough stem biomass to perform the extraction with 4.0 grams; however, the leaf samples contained very minimal biomass thus the extraction was performed with a biomass ranging from 0.7 - 2.0 grams. Using a ratio of water for the corresponding mass, the concentrations to be extracted were normalized. In Appendix A.3, the raw data for the extraction portion of the procedure can be seen.

From the extracted samples, an average of 91% of solution for stem samples and 82% of solution for leaf samples were recovered following the vacuum filtration. These samples were calculated based on the initial deionized water added and the volume of solution collected. These values do not account for the extracted mass from the plant.

Using the mass of the biomass pre- and post-extraction, the team could infer the maximum amount of plant biomass that was potentially extracted in the solution. This is a maximum percentage due to the fact that some plant biomass would be lost during the blending of the water and biomass, and some of the biomass would be lost during the collection after the filtration occurred. Below is a table of the maximum amount of biomass extracted based on sample and plant component.

Table 6: Extractions for Leptoplax Samples

Sample	Max Leaf Extracted	Max Stem Extracted
L2	41.10%	21.32%
L3	32.19%	18.68%
Average	36.64%	20.00%

The L2 samples were averaged to produce the values shown in the leaf and stem section, and the same was done for the L3 samples. During the mixing phase, the team observed that more of the organic material within the leaves was extracted into the solution than the stems. This could be due to the physical characteristics of the plant component. Also, it is noted that overall, the organic material in the L2 leaf and stem samples were extracted at a higher rate than the L3 samples. This data can be seen in Appendix A.4. However, no definitive conclusions can be drawn due to the limited sample size. Also, the methods used during these experiments were inconsistent thus multiple trials would need to be performed to draw definitive conclusions.

4.1.2. Alyssum *murale* Samples

Following the procedures for sample preparation, Alyssum *murale* Co-Culture Plus (CC+) and Co-Culture (CC) samples were prepared during the week of January 23rd. Two plants from the CC+ sample selection and two plants from the CC selection were gathered. The leaves and stems of each plant were separated. The plant components were ground and the loss of material was measured. In Appendix B.1, the raw data for the collection portion of the procedure can be seen, and in Appendix B.2, the raw data for the grinding portion of the procedure can be seen. The average loss of plant material during this step was 5.38%. The material loss values for each plant sample can be seen in the table below.

Table 7: Material Loss for Alyssum Samples

Sample	Material Loss Percentage
1 - CC+ Stem	0.89%
1 - CC+ Leaf	5.32%
2 - CC+ Stem	0.12%
2 - CC+ Leaf	4.14%
1 - CC Stem	3.68%
1 - CC Leaf	20.14%
2 - CC Stem	1.45%
2 - CC Leaf	7.33%

As discussed earlier in *4.1 Sample Preparation,* the team attempted to keep the material loss values relatively low; however, total material loss was inevitable.

Mass balances of each plant sample were conducted with their post-grinding mass. Below is a table of each sample's mass balance.

Table 8: Mass Balance for 1-CC+

1 - CC+			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	20.11	24.41	82.38%
Leaf	4.3		17.62%

Table 9: Mass Balance for 2-CC+

2 - CC +			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	8.46	12.97	65.23%
Leaf	4.51		34.77%

Table 10: Mass Balance for 1-CC

1 - CC			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	11.79	16.39	71.93%
Leaf	4.6		28.07%

Table 11: Mass Balance for 2-CC

2 - CC			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	15.67	17.82	87.93%
Leaf	2.15		12.07%

Based on the calculations, the plant's biomass is mostly comprised of stem material with an average of 73.81wt% for CC+ and 79.93wt% for CC. The averages for leaf biomass were 26.19wt% and 20.07wt% for CC+ and CC, respectively. The biomasses of the specific plant components were used to normalize the final nickel concentration from each section of the plant. This normalization allowed the team to draw more definitive conclusions on what section of the plant provides a higher extracted concentration of nickel.

From the collected ground material, there was enough stem biomass to perform the extraction with 4.0 grams; however, two of the leaf collections, 1-CC+ and 2-CC, did not produce enough material to perform an extraction with 4.0 grams thus 2.0 and 1.0 grams were used for the samples, respectively. Using a ratio of water for the corresponding mass, the concentrations to be extracted were normalized. In Appendix B.3, the raw data for the extraction portion of the procedure can be seen.

From the extracted samples, an average of 92% of solution for stem samples and 90% of solution for leaf samples were recovered following the vacuum filtration. These samples were calculated based on the initial deionized water added and the volume of solution collected. These values do not account for the extracted mass from the plant.

Using the mass of the biomass pre- and post-extraction, the team could infer the maximum amount of plant biomass that was potentially extracted in the solution. Below is a table of the maximum amount of biomass extracted based on sample and plant component.

Table 12: Extractions for Alyssum Samples

Sample	**Max Leaf Extracted**	**Max Stem Extracted**
CC+	42.43%	29.30%
CC	44.75%	26.65%
Average	43.64%	27.77%

The CC+ samples were averaged to produce the values shown in the leaf and stem section, and the same was done for the CC samples. During the mixing phase, the team observed that more of the organic material within the leaves was extracted into the solution than the stems. This could be due to the physical characteristics of the plant component. The CC+ stem samples performed better than the CC stem samples, but the reverse occurred for the leaf samples. However, when looking at the individual data, the CC samples tend to have a wider range for the values, 21.5% - 31.0% for stem 39.5% - 50.0% for leaf, compared to CC+, 28.3% - 30.4% for stem and 40.5% - 44.6%

for leaf. This data can be seen in Appendix B.4. No definitive conclusions can be drawn due to the limited sample size. Also, the methods used during these experiments were inconsistent thus multiple trials would need to be performed to draw definitive conclusions.

4.1.3. Comparison of Leptoplax *emarginata* and Alyssum *murale* Sample

Comparisons of stem and leaf samples and plant species can be made with the data compiled during the sample preparation phase for samples L2, L3, CC+, and CC. Using the observations, data and calculations for mass balances and plant extractions, physical differences can be seen. These comparisons disregard the material loss portion because material loss is due to human error and has nothing to do with the plant itself. Also, it is to be noted that this section does not compare the biochemical makeup of the plant. Those comparisons will be discussed in the *Sample Analysis* section in **Results & Discussion**.

Below are images of a Leptoplax *emarginata* and Alyssum *murale* plant.

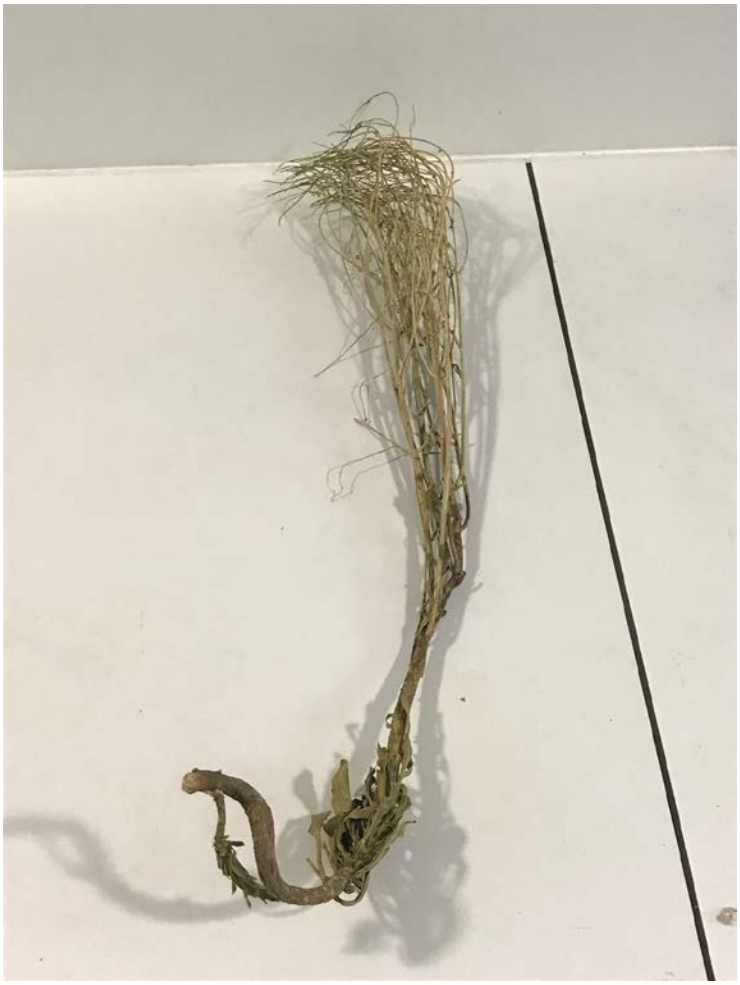

Figure 3: Leptoplax emarginata

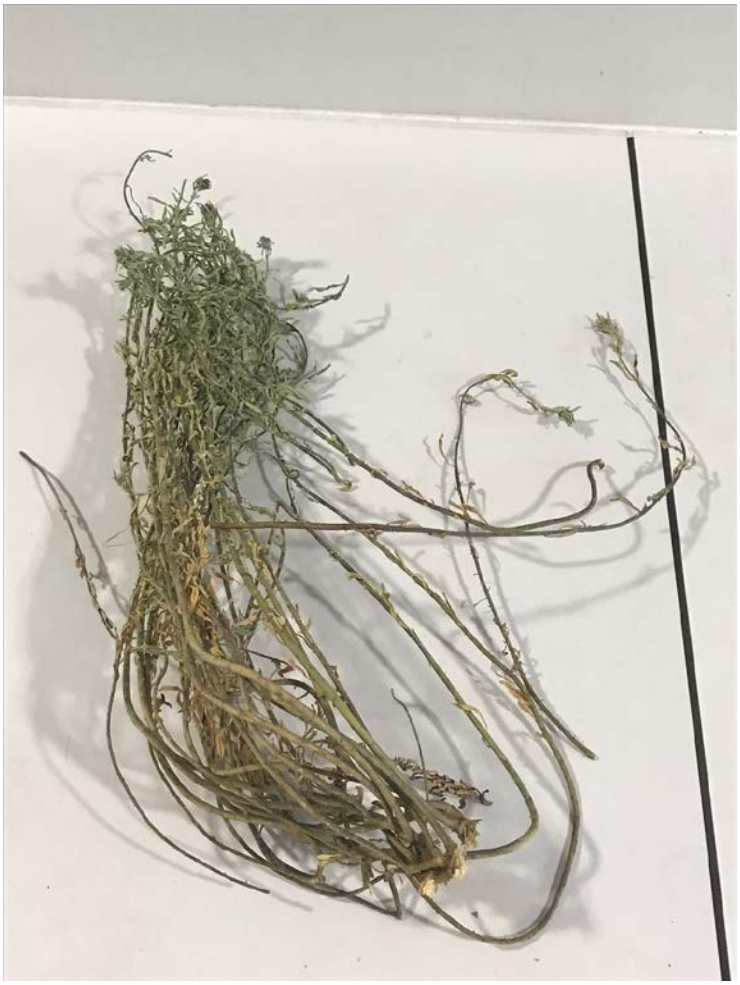

Figure 4: Alyssum murale

From the images, physical differences can be seen between the plants. The Leptoplax plant originates from one singular stem with few minor branches near the top of the plant; however, the Alyssum plant has multiple major stems branching off from the root. It also appears that the Alyssum plant produces more leaves than the Leptoplax plant. Both plants have similar texture regarding their leaves and stems.

In regards to mass balances, the values for seeds and flowers of the L2 and L3 samples were neglected to provide a better comparison of each plant species because CC and CC+ did not produce any flower or seed samples. Below are the updated mass balances of the L2 and L3 samples when the flower and seeds were not taken into account.

Table 13: Mass Balance for 1-L2

1 - L2			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	25.82	29.72	86.88%
Leaf	3.9		13.12%

Table 14: Mass Balance for 2-L2

2 - L2			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	11.84	13.46	87.96%
Leaf	1.62		12.04%

Table 15: Mass Balance for 1-L3

1 - L3			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	33.54	35.56	94.32%
Leaf	2.02		5.68%

Table 16: Mass Balance for 2-L3

2 - L3			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	41.11	41.69	98.61%
Leaf	0.58		1.39%

On average, the L2 samples are 87.42wt% and 26.19wt% stem and leaf, respectively. On the other hand, L3 samples are 96.46wt% and 3.54wt% stem and leaf, respectively. CC+ samples on average are 73.81wt% stem and 26.19wt% leaf, and CC samples are on average 79.93wt% stem and 20.07wt% leaf.

When comparing these mass balances to the Alyssum samples shown in the section *Alyssum murale Samples*, it appears that the Alyssum samples have a more even distribution of leaf and stem masses; however, the Alyssum samples are still more stem mass dominant. These values correlate to the physical descriptions of each plant, with more stems being collected from the Alyssum samples than the Leptoplax samples. Also, leaves are not as heavy as the stems thus with a higher quantity of stems being collected means that it will account for a higher percentage of the overall plant mass. The Leptoplax samples did not produce a high quantity of leaves thus this accounts for the low weight percent.

Referring to sections *Leptoplax emarginata Samples* and *Alyssum murale Samples* and Appendices A.3 and B.3, the extraction data for the two plant species can be seen. From the raw and analyzed data, the team observed that the Leptoplax samples are more readily extracted into the solutions for both stem and leaf extraction experiments compared to the Alyssum samples. When comparing the plant component of each respective sample, it can be inferred that leaves are more readily extracted into solution than stem samples. On average, 27.77% and 43.64% of the Leptoplax stem and leaf biomasses, respectively, are extracted into the solution. On the other hand, on average, 20.0% and 36.64% of the Alyssum stem and leaf biomasses, respectively, are extracted into the solution. This could be attributed to a variety of reasons such as: plant biology or the operation of

the procedure. However, no definitive conclusions can be inferred due to the small sample size, and further trials would need to be conducted to draw definitive conclusions.

4.1.4. Re-Runs of Leptoplax *emarginata* & Alyssum *murale* Samples

Due to a complication of nickel results obtained from the CC+ samples conducted in the *Sample Analysis* phase, new sample preparations had to be performed. Following the procedures for sample preparation, L2, L3, CC+, and CC samples were prepared during the week of February 2nd. One plant for each of the species sample, L2, L3, CC and CC+, were taken and crushed without the separation of leaves and stems. However, an extra CC+ plant was taken and separated by leaves and stems. The plants and plant components were ground and the loss of material was measured. In Appendix C.1, the raw data for the collection portion of the procedure can be seen, and in Appendix C.2, the raw data for the grinding portion of the procedure can be seen. The average loss of plant material during this step was 5.56%. The material loss values for each plant sample can be seen in the table below.

Table 17: Material Loss for Re-Run Samples

Sample	Material Loss Percentage
3 - L2 Whole	22.00%
3 - L3 Whole	7.88%
3 - CC Whole	0.64%
4 - CC+ Whole	1.03%
3 - CC+ Stem	1.42%
3 - CC+ Leaf	0.38%

As discussed earlier in *4.1 Sample Preparation,* the team attempted to keep the material loss values relatively low; however, total material loss was inevitable.

Mass balances were not conducted for the whole plant samples because there was no separation performed. However, a mass balance for the 3-CC+ sample was performed.

Table 18: Mass Balance for 3-CC+

3 - CC+			
	Mass (g)	**Total Mass**	**Wt. Percentage of Plant**
Stem	15.85	27.66	57.30%
Leaf	11.81		42.70%

Based off the calculations for mass balance for this CC+ sample, it can be inferred that the stems comprise the majority of the plant, which is similar to the other CC+ samples. However, 3-CC+ had a higher weight percent of leaves than the other Alyssum samples. This is due to the variation between each plant and how it matures.

From the collected material and ground material, there were enough stem and leaf biomasses to perform the extractions with 4.0 grams. In Appendix C.3, the raw data for the extraction portion of the procedure can be seen.

From the extracted samples, an average of 91% of solution for the whole samples, 84% of the solution for the stem sample for 3-CC+, and 95% of solution for the leaf sample for 3-CC+ were recovered following the vacuum filtration. These samples were calculated based on the initial deionized water added and the volume of solution collected. These values do not account for the extracted mass from the plant.

Using the mass of the biomass pre- and post-extraction, the team could infer the maximum amount of plant biomass that was potentially extracted in the solution. Below is a table of the maximum amount of biomass extracted based on sample and plant component.

Table 19: Extractions for Whole Plant Samples and 3-CC+ Sample

Sample	Max Whole Plant Extracted	Max Leaf Extracted	Max Stem Extracted
L2	19.70%		
L3	29.18%		
CC	36.63%		
CC+	30.42%		
3-CC+		40.15%	30.50%
Average	28.98%		

Based on the max extracted data, the Alyssum organic matter is extracted more readily into the solution, which correlates to the previous experiments conducted on Leptoplax and Alyssum. In the first trial, the organic matter in Alyssum samples was extracted into the solution more readily than the Leptoplax samples. Also, from this data it can be seen that the organic matter in the leaves of the CC+ sample was more readily extracted than the stem, which correlates to the trials run earlier. This data can be seen in Appendix C.4. However, no definitive conclusions can be drawn due to the limited sample size. Also, the methods used during these experiments were inconsistent thus multiple trials would need to be performed to draw definitive conclusions.

4.2. Sample Analysis

There were three main goals the team sought to achieve during the *Sample Analysis* portion: characterize and identify the organic compounds in solution, determine nickel concentration in solution, and to determine the total organic carbon and nitrogen concentration in solution. This portion of the **Results and Discussion** section will be organized by the stated goals.

4.2.1. Characterization and Identification of Organic Compounds in Solution

During the characterization and identification the organic compounds in solution, the *UV-Visible Spectroscopy* and *Fluorescence Spectroscopy* methods were utilized. These methods allowed for the team to identify humification and biological indexes, which were then utilized to determine the fluorophores of the organic material. In their respective sections, the results are discussed.

4.2.1.1. Fluorescence Indexes

Following the procedures described in the **Methodology** section for the characterization of organic compounds in solution, the team analyzed the results of the fluorescence spectroscopy test and calculated the appropriate fluorescence indexes.

Humification Index (HIX)

In order to better understand the organic chemistry of the agromining extract, the team calculated the humification index of each sample. The index is used in order to determine the degree of humification of dissolved organic matter in river samples. This provides a gauge for the complexity of the aromatic compounds of a solution since an increase in humification is associated with an increase in the carbon to hydrogen (C/H) ratio. Fluorescence spectroscopy can detect this process because a rise in the C/H ratio will result in shift to longer wavelengths of the peak emission (Ohno, 2002). Although HIX is most commonly used when characterizing natural water samples, where stages of humification vary throughout a water body, it may be applied in this experiment to provide insight into the complexity of the aromatic compounds and the concentration of the organic matter in the plant components.

Prior to testing, the team anticipated that the HIX results would indicate that the DOM present in the sample solutions was non-humified. This is because humification is a result of organic decomposition, and by freezing/refrigerating our samples, very little or no decomposition should have occurred. With the equation the team implemented, a high degree of humification would be represented by an HIX value of 0.9 or greater, a moderate degree of humification represented by a HIX value of 0.85 or greater, and no humification represented by a value of 0.57 or less (Ohno, 2002). After calculating the HIX for each sample, the initial assumption was confirmed by the HIX values ranging from 0.1647 to 0.5647. HIX values in this range indicate the presence of non-humified, carbon-rich, highly water soluble organic matter. With the degree of humification being confirmed, the team was then able to use the HIX index to analyze the aromatic complexity of the DOM. The HIX results for samples, which were separated by leaf and stem, may be seen in the figure below.

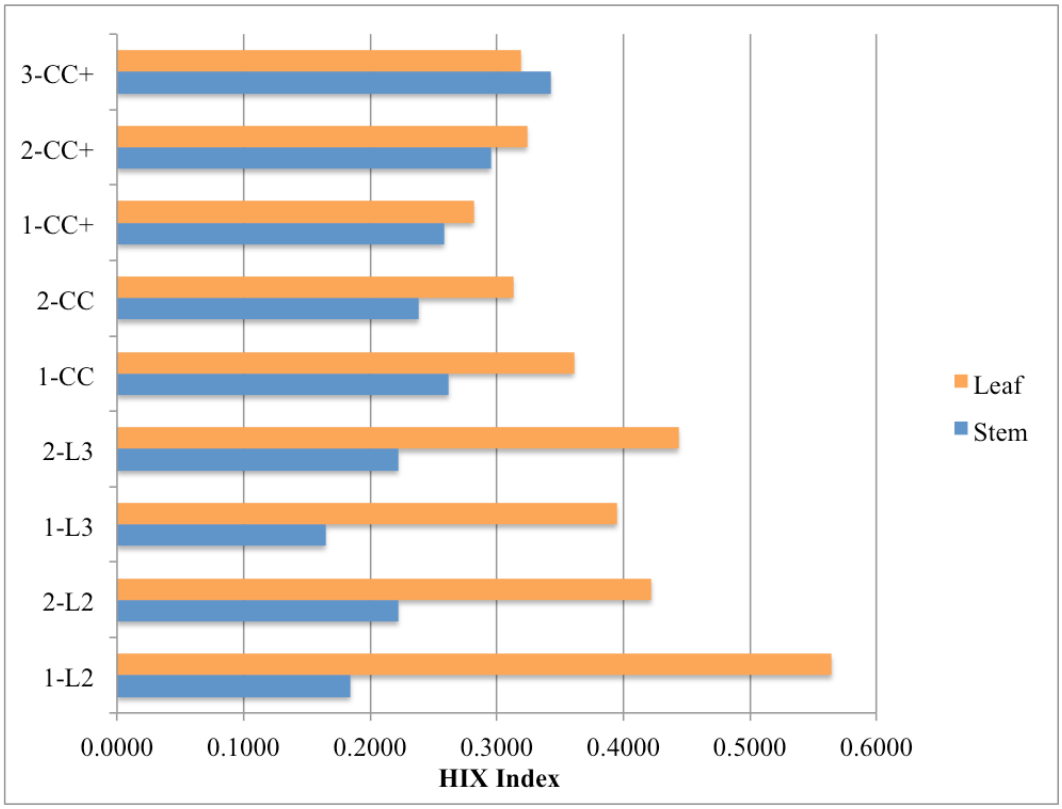

Figure 5: Humification Index Results of Leptoplax emarginata and Alyssum murale

Comparison of the HIX results of each leaf sample to the respective stem sample demonstrated that the organic matter within the leaves of both plant species is more complex, or contains higher molecular weight aromatics, than the stems. This is indicated by a consistently higher HIX value for the leaf samples. The variance in the HIX values between the leaves and stems may be attributed to the manner in which organic compounds are transported and utilized within a plant during its life cycle. The cellular structures in a leaf are more intricate and job specific than the structures in the stem, requiring more complex compounds.

When comparing the organic matter between species, the team found that Leptoplax leaves had a greater HIX value than Alyssum leaves, while Alyssum stems had a greater HIX value than Leptoplax stems. With a greater aromatic complexity in the leaves of than Alyssum, agromining nickel without ashing may prove more difficult for Alyssum depending on the identity and characteristics of the organic compounds. Furthermore, the team determined that the method of Alyssum cultivation had no impact on the HIX index.

The implication of this is that with both species storing higher weight organic compounds and nickel within the leaves, recovering nickel from the leaves will require organometallic complexes to be broken that are possibly stronger than those formed between nickel and stem material. The team determined that the organic compounds detected through the HIX index are glucosinolate, a form of indole, and humic-like substances. The team came to discover these compounds via a synchronous-50 fluorescence test; their implications will be discussed in *Identification Organic Compounds in Solution* section.

Biological Index (BIX)

The team used the biological index in combination with the humification index to determine the relative age of the DOM in the plant extract solutions. In natural water samples, a high BIX value, greater than 0.7, corresponds to recent biological activity and is detected by the presence of β fluorophore (Huguet et al., 2009). Although, the team expects there to be very little autochthonous activity, a comparison of the index values, to assess the age of the organic matter, can still be conducted.

All calculated BIX values are considered to be low with results ranging from 0.127 to 0.565, supporting the low autochthonous activity assumption. The BIX results for the samples, which were separated by leaf and stem, may be seen in the figure below.

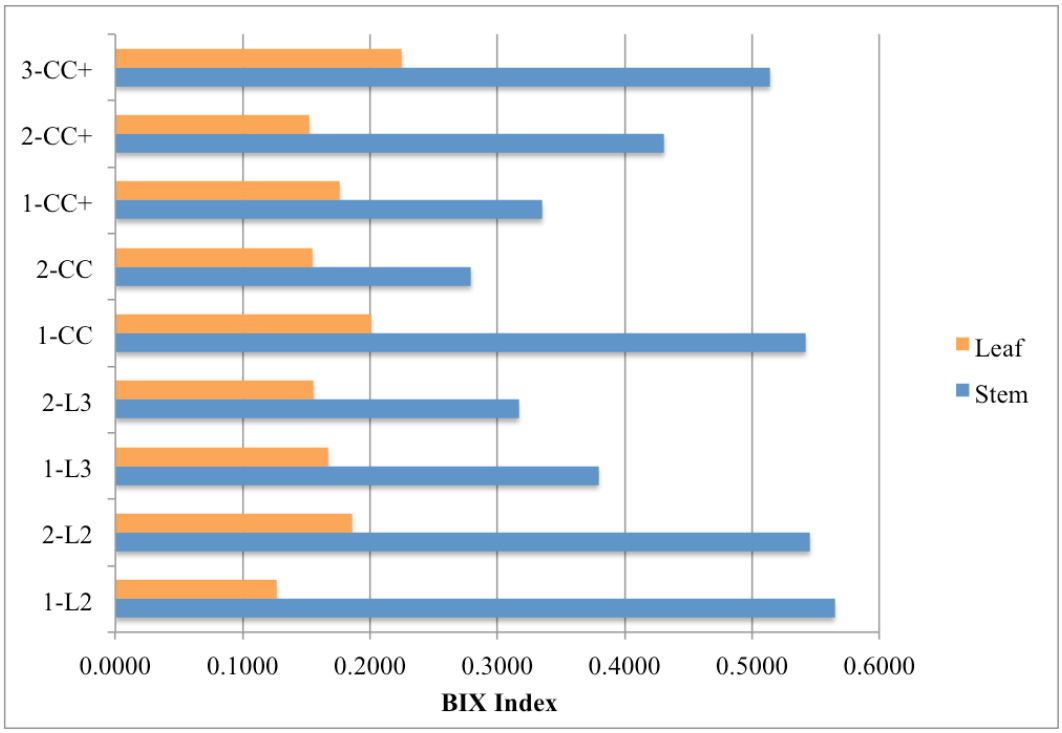

Figure 6: Biological Index Results of Leptoplax emarginata and Alyssum murale

Based on the BIX results, it is apparent that the organic matter in the stems of Leptoplax and Alyssum was formed more recently than the organic matter in the leaves. Using an average of the BIX values, the team calculated the ratio of stem: leaf and found that it was 2.84 and 2.31 for Leptoplax and Alyssum, respectively. With such a large difference between the BIX values of the stems compared to the leaves, the team determined that there is a distinct difference between the relative age of the DOM in the stems and leaves in both species. This conclusion is supported by the HIX results since the HIX results for leaf samples are higher than the stems, indicating that the leaves contain more complex aromatics. We can presume that the detected organic complex gain complexity with time and thus we find more complex compound in areas with greater DOM age. In addition, when taking into account the manner in which nutrients and organic material transported, discovering more recently produced organic material in the stems is expected.

As stated in the discussion of the HIX index, the implication of having more complex and now older organic material in the leaves of hyperaccumulators is that there may be stronger bonds formed between the organic compounds and nickel. In addition, the team was unable to come to a definite distinction between the relative age of the Leptoplax and Alyssum.

Fluorescence Index (FI)

The final index the team used to characterize the agromining extract was the fluorescence index. Similar to the previously described indexes, the fluorescence index is traditionally used when describing natural water bodies in order to differentiate aquatically/microbially derived DOM from terrestrially derived DOM (Mcknight et al., 2001). An index value of 1.9 is representative of aquatic or microbially sourced DOM while a value of 1.3 is indicative of soil being the primary source of the DOM. As our samples are not river samples, the team will be using the index to draw conclusions about the organic matter that fluoresces with an excitation wavelength of 370 nm, and how it may differentiate between plant components The FI index results may be seen in the figure below.

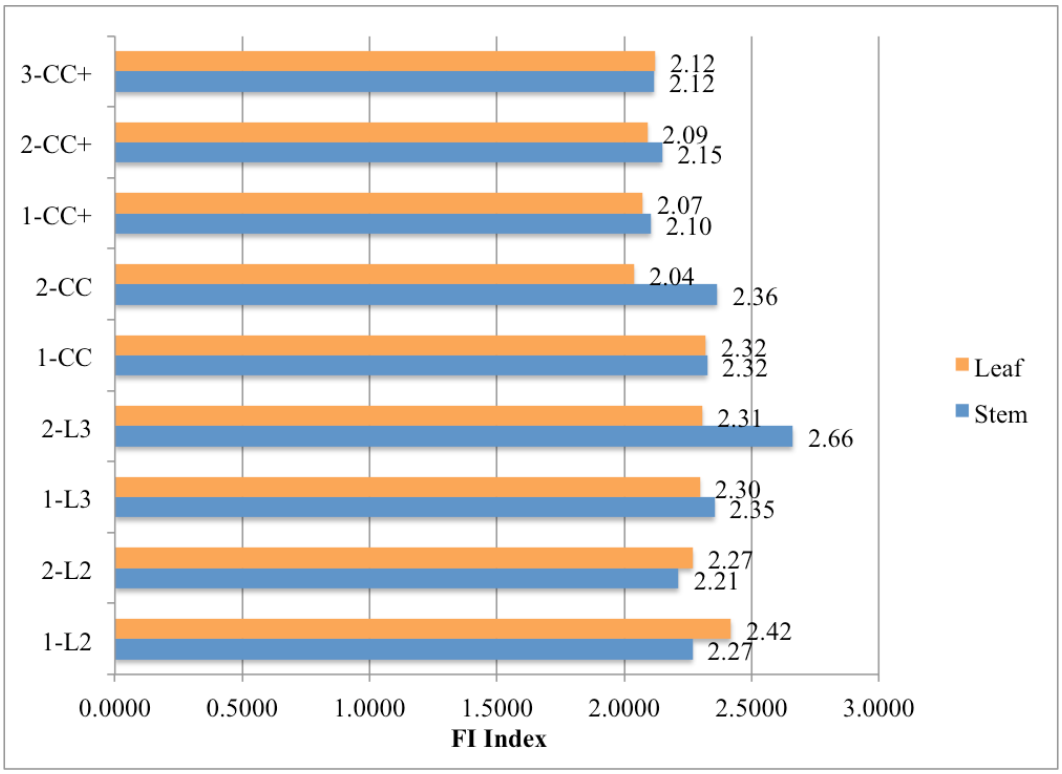

Figure 7: Fluorescence Index Results of Leptoplax emarginata and Alyssum murale

The fluorescence index results for all samples are within a small range of each other but, with this test, variations in results of 0.1 indicate a difference in the characteristics of the organic matter (Mcknight et al., 2001). The general trend is that there is no significant difference between the organic matter, which fluoresces at this wavelength in the stems, and leaves of each species. This trend is not upheld with the results from 1-L2, 2-L3 and 2-CC. Further analysis revealed that there is a notable difference between the organic matter within Leptoplax and Alyssum. With no clear

indication to what type of organic matter is represented by the FI results, the team was unable to discuss the implications of this test on the removal of nickel.

4.2.1.2. Identification of Organic Compounds in Solution

After the synchronous-50 results were corrected for the inner-filter effect using the UV-visible absorbance spectra data, they were run through the dissolution program, allowing the team to determine that there are four fluorophores present in both the Leptoplax and Alyssum extract samples. The results of the dissolution program may be seen in Appendix D.1. An example of the spectra for all whole plant samples may be seen in Figure 8, the peaks signify the detected fluorophores. It must be noted that the team did not analyze the whole plant samples because there is no way to ensure the sample was an accurate representation of the leaf to stem ratio of the plant.

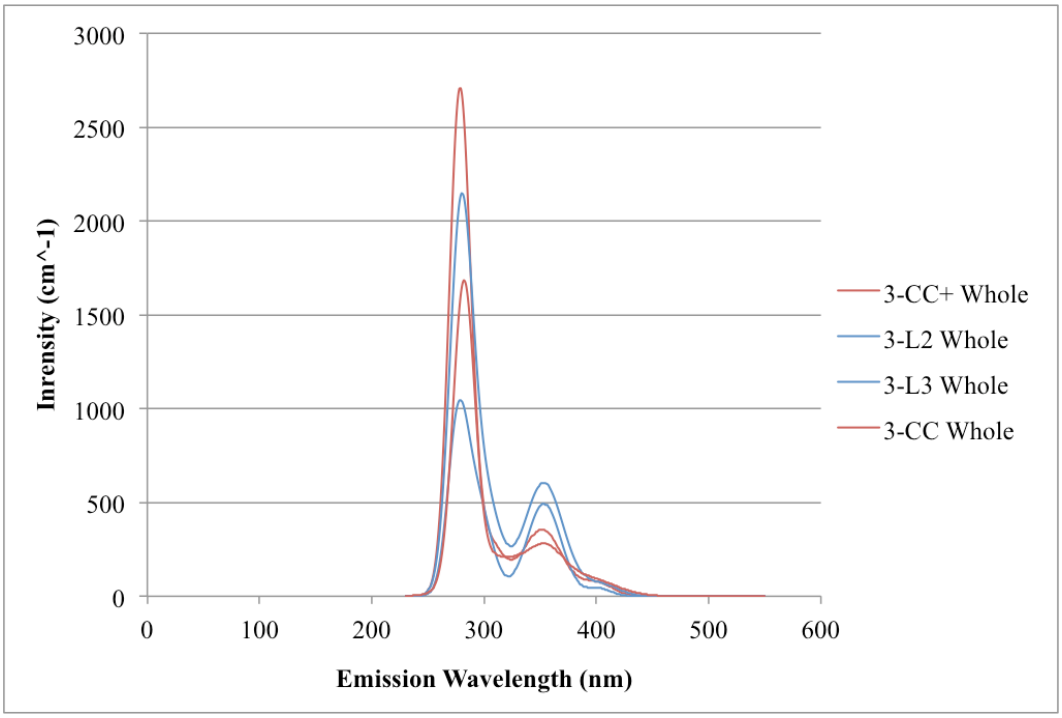

Figure 8: Synchronous-50 Fluorescence Spectra

The large intensities of two of the fluorophores prevented the team's ability to clearly see that there are in fact four fluorophores present in the solutions. The dissolution program was able to identify the emission wavelengths of the fluorophores and with this information the team was able to identify the organic compounds, which they likely represent. The wavelengths at which the fluorophores emitted photons and which compound they represent may be seen in the table below.

Table 20: Leptoplax and Alyssum Extract Fluorophores

Fluorophore #	Emission Wavelength (nm)	Compound
1	280	Indole Group Attached to Glucobrassicin
2	300	Possibly a Component of Glucobrassicin
3	355	Humic Substance
4	400	Humic Substance

With the same sample concentrations being used when preparing the plant extracts, the team was able to compare the intensities for each sample and correlate an increase in intensity to an increase in fluorophore concentration.

Fluorophore 1 and 2

Reading the spectra from short to long wavelengths, the first fluorophore identified had an approximate emission wavelength of 280 nm among all Leptoplax and Alyssum samples. The second fluorophore had an approximate emission wavelength of 300 nm. Fluorophore 1 intensities of each sample maybe seen in the Figure 9 and fluorophore 2 intensities may be seen in Figure 10.

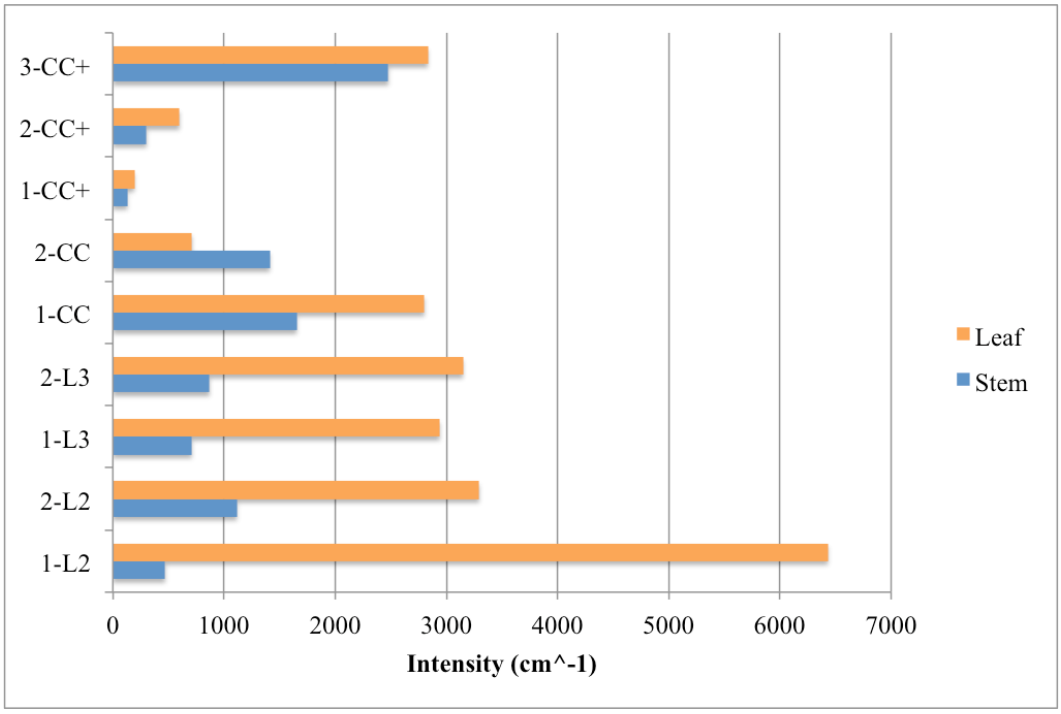

Figure 9: Fluorophore 1 Intensity of Leptoplax and Alyssum (λ_{em}= 280 nm)

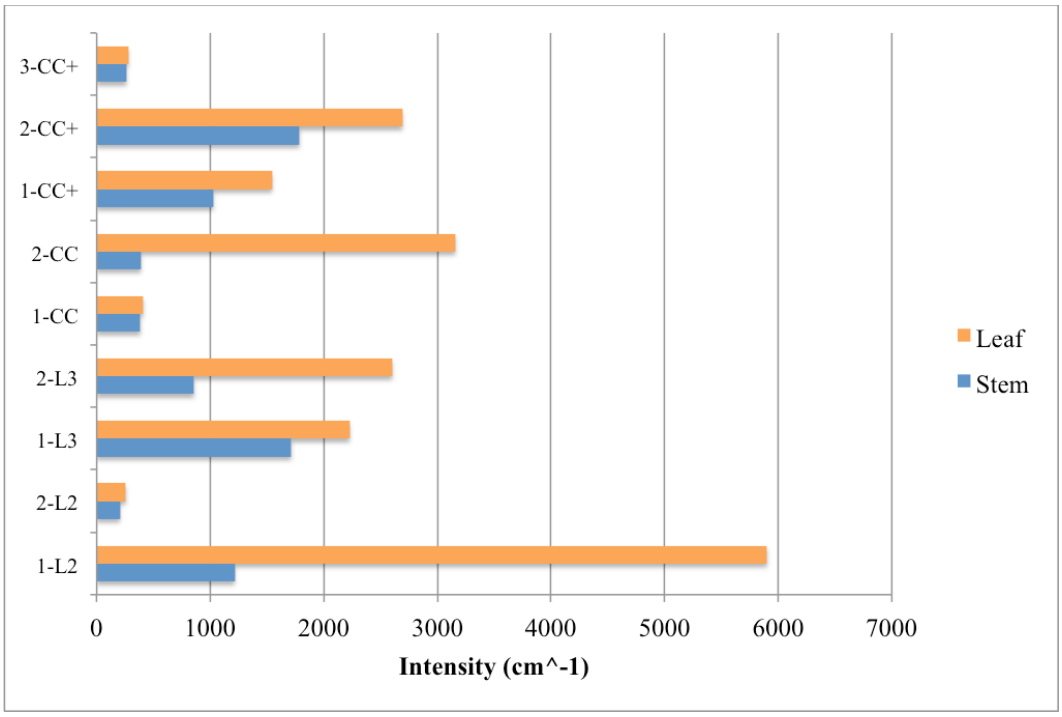

Figure 10: Fluorophore 2 Intensity of Leptoplax and Alyssum (λ_{em}= 300 nm)

Fluorescence at 280 nm is indicative of a variation of an indole compound, which the team expects is attached to the side chain of the organic compound glucosinolate. The two compounds form the more complex organic aromatic, glucobrassicin. Glucosinolate is a common organic compound present in plants of the brassicaceae family, such as Alyssum *murale* and Leptoplax *emarginata*. This organic compound is known to complex with indole when it is present; leading the team to believe glucobrassicin is in the agromine extract solution. The team identified the indole group through comparison to known tryptophan spectra values since indole is a building block of tryptophan. With the fluorescence peaks of our samples and those of tryptophan appearing at similar emission wavelength, the team can conclude the peak represents an indole compound. Prior to the team's study, there was the belief that indole was present in the extract solutions and impacting the removal of nickel. By confirming the presence of indole, further research may be done with the focus on the affinity for nickel to complex with indole.

The team calculated the average intensity ratio of leaves: stems to be 4.99 for Leptoplax and 1.19 Alyssum. This indicates that the leaves of each plant are more concentrated in indole, and in turn glucobrassicin, than their respective stems. This is supported by the studies that reveal glucosinolate is primarily stored in plant vacuoles located in the seeds and leaves of brassicaceae plants, with some glucosinolate stored in stem cells (Redovnikovic, 2008). When comparing the ratio of Leptoplax leaves to Alyssum leaves and Leptoplax stems to Alyssum stems, the team found that the ratios were approximately 2.7 and 0.67, respectively. This indicates that Leptoplax leaves are more concentrated in indole than Alyssum leaves; however, based on the team's samples, Alyssum steams are more concentrated in indole than Leptoplax.

The team believes that fluorophore 2 is also indicative of a component within the glucosinolate compound. More research should be done in order to determine whether this is in fact true, as the team did not observe the same trends for fluorophore 1. The variation in the

trends between the two fluorophores may be due to the fact that not every glucosinolate compound will complex with indole, causing there to be a difference in intensities between the two, as fluorophore 1 specifically represents indole.

Whether fluorophore 2 represents glucosinolate or not, the compound is more concentrated in the leaves of both species. The ratio of Leptoplax leaves to stems and Alyssum leaves to stems is 2.8 and 1.6, respectively. In addition, the team's results indicate that each component of Leptoplax is more concentrated in the compound than each component of Alyssum. The team cannot confirm whether fluorophore 2 represents glucosinolate due to the variations between the results of fluorophore 1 and 2. Although the concentration of fluorophore 2 and 1 were greater in the leaf samples for both species than their respective stems, when comparing the value of the average intensity ratios of leaves to stems, the ratios between the two fluorophores were not similar. Also to be noted, the ratios between Leptoplax leaves to Alyssum leaves and Leptoplax stems to Alyssum stems are 1.7 and 1.3, respectively. These do not correspond to fluorophore 1 where the ratios were 2.8 and 0.7. With this difference in ratios between fluorophore 1 and 2, it is not definite whether fluorophore 2 is a component of glucosinolate.

The team has confirmed the presence of an indole group, which the team believes to be attached to the compound glucosinolate. Although, the team were unable to confirm whether fluorophore 2 did indeed represent glucosinolate, it is believed it does and that the variation in intensities between fluorophore 1 and 2 are because is not reasonable to assume every glucosinolate compound complexes with indole. However, the team was unable to draw a clear correlation between fluorophore 1 or 2 concentrations and nickel extracted. This will be discussed further in section *Determination of Metal Concentrations.*

Fluorophore 3 and 4

The third and fourth fluorophore in the plant extract solutions fluoresce at an emission wavelength of approximately 355 and 400 nm, respectively. In river samples, peaks at the mentioned wavelengths would indicate the presence of humic like substances. Although, the tested samples were not river samples, the peaks do represent organic compounds. The intensity results of fluorophores 3 and 4 may be seen in the figures below.

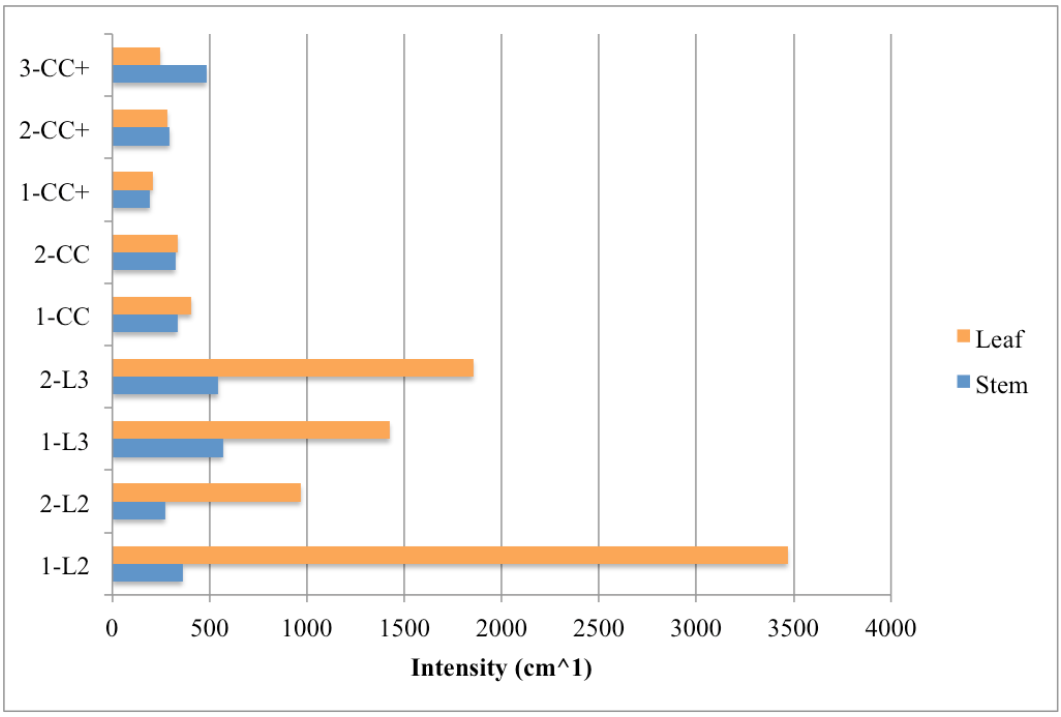

Figure 11: Fluorophore 3 Intensity of Leptoplax and Alyssum (λ_{em}= 355 nm)

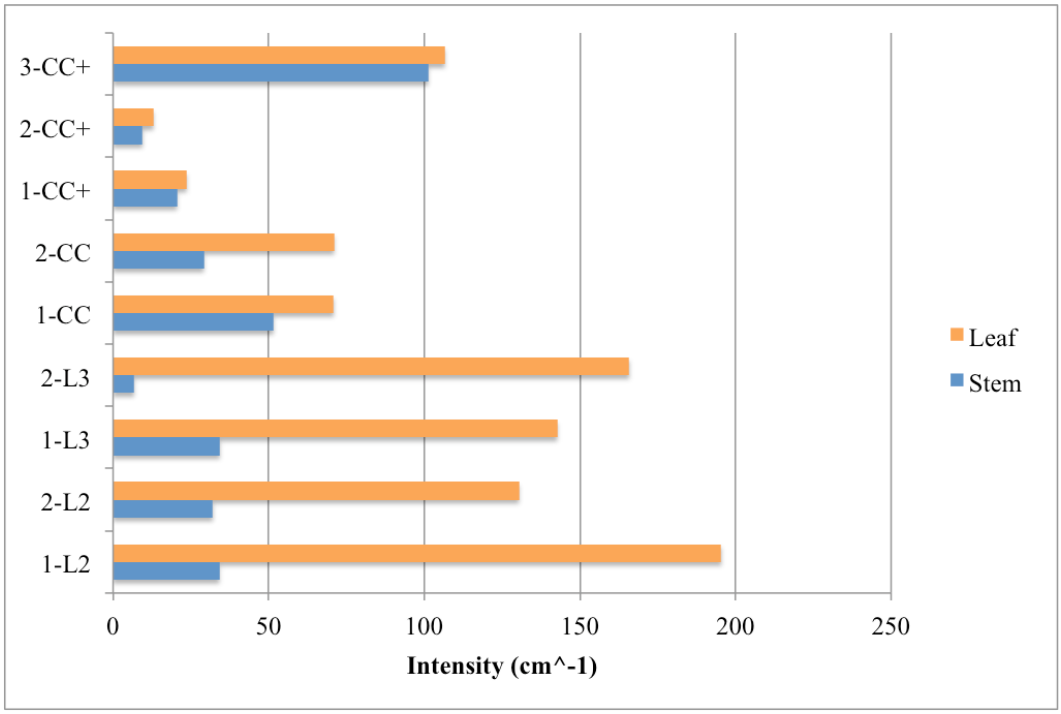

Figure 12: Fluorophore 4 Intensity of Leptoplax and Alyssum (λ_{em}= 400 nm)

Analysis of the fluorophore 3 results show that Leptoplax leaves are consistently more concentrated in the organic compound represented by the fluorophore. Leptoplax leaves had an average concentration of fluorophore 3 that was 4.4 times greater than the stems, demonstrating the drastic difference between leaves and stems of Leptoplax. When comparing Alyssum leaves

to their respective stems, the team noted a different trend than that in Leptoplax. The concentration of the organic compound fluorophore 3 represents is nearly the same in both the leaves and stems of Alyssum, with a 0.9 ratio of leaf to stem. However, there is a notable difference between the concentrations of the compound in each species of hyperaccumulator. The team found that Leptoplax leaves were 6.5 times more concentrated in the organic compound than Alyssum leaves and 1.3 times more concentrated when comparing stems. In order to identify the compound represented by fluorophore 3, research should be done on organic compounds that are present in both species, but with a focus on compounds more concentrated in Leptoplax leaves than Leptoplax stems and similarly concentrated throughout an Alyssum plant.

Based off our analysis of fluorophore 3 and 4, the team has concluded that the two fluorophores represent separate organic compounds because the trends described above in regards to fluorophore 3 are not similar to those of fluorophore 4. Although, the concentration of fluorophore 4 in Leptoplax leaves is greater than the stems, the magnitude by which leaves are more concentrated is significantly greater for fluorophore 4 than 3. In this case, the fluorophore 4 ratio of leaf to stem intensity being 5.9 compared to the 4.4 of fluorophore 3. The team observed a similar finding when comparing the fluorophore 3 and 4 intensity ratios for Leptoplax leaf to Alyssum leaf. Leptoplax leaves were more concentrated than Alyssum leaves in fluorophore 4, similar to fluorophore 3, but the magnitude by which Leptoplax leaves were more concentrated than Alyssum leaves varied between the two fluorophores. The team found the intensity ratio of Leptoplax leaves to Alyssum leaves to be 2.8 in fluorophore 4, compared to the much larger ratio of 6.5 calculated for fluorophore 3. In addition, Alyssum leaves are also more concentrated in fluorophore 4 than the stems, which directly oppose the trend the team found when analyzing fluorophore 3. Furthermore, the team noted the apparent difference in concentrations of fluorophore 4 between Alyssum leaves and stems, while there was little difference noted when analyzing concentrations of fluorophore 3. With this information, the team concluded that fluorophore 4 represents a different organic compound than fluorophore 3. To identify this compound research should be done with the aim to find an organic compound that is constantly more concentrated in Leptoplax and Alyssum leaves, but is highly concentrated in Leptoplax leaves.

The team was unable to determine the identity of the compounds which fluorophore 3 and 4 represent and more research should be done to identify these compounds. In addition, the team was unable to draw any clear correlations between the concentrations of fluorophore 3 and 4, and nickel extracted into the solution. This will be discussed further in section *Determination of Metal Concentrations in Solution.*

4.2.2. Determination of Metal Concentrations in Solution

For the solution to be analyzed by the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) machine, the team had to prepare the solution through the *Mineralization* procedure, which can be seen in the **Methodology** section. All samples except for the whole plant samples were analyzed using ICP-AES to determine their mineral content. As discussed in the **Methodology** section, the mineralization process destroys all organic material present in the solution while the ICP-AES allows for the identifications of metallic elements in the solution. This was useful for the team because this analysis could determine the concentration of the nickel extracted into the solution, some of which were complexed with the organic material. Also, this

information may be used to draw conclusions between the identified organic compounds and their affinity to complex with nickel. This procedure only identifies the metal elements in the plant after the organic material is destroyed thus it is important to identify those compounds to attempt to separate the nickel out from the organic matter is complexes with.

4.2.2.1. Leptoplax emarginata Samples

The Leptoplax samples, 1-L2, 2-L2, 1-L3, and 2-L3, were first analyzed separately from the Alyssum samples to determine the differences between the Leptoplax samples themselves. Below is a chart detailing the overall concentrations of nickel in the plant samples.

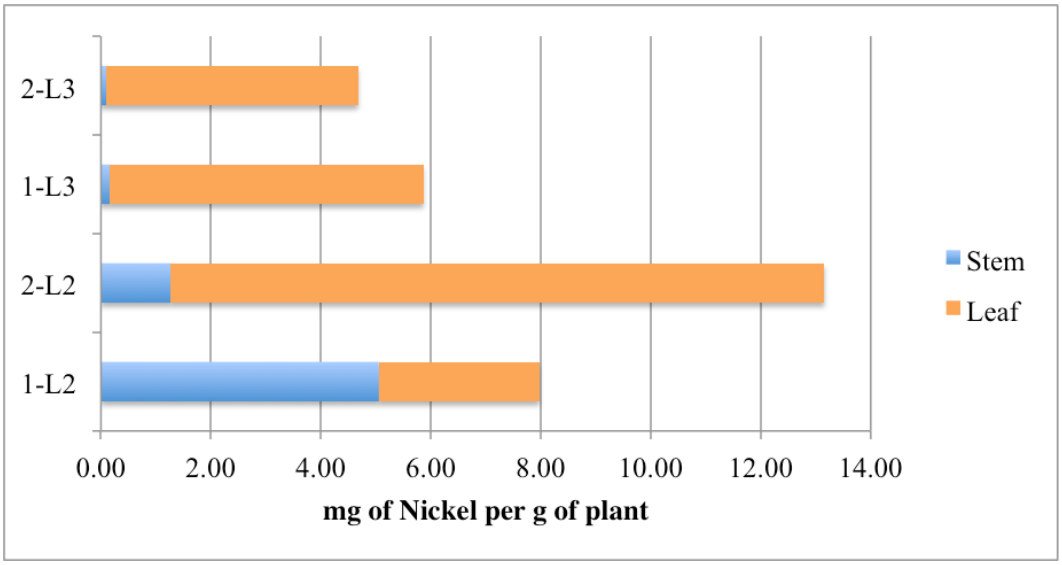

Figure 13: Nickel Concentrations in Leptoplax Samples

The team observed that L2 samples had a higher nickel extraction than L3 samples by there being more nickel per mass of plant material. L2 samples produced 5.05 mg/g and 1.27 mg/g of nickel from stems and 2.92 mg/g and 11.89 mg/g of nickel from leaves compared to the 0.16 mg/g and 0.10 mg/g of nickel from stems and 5.7 mg/g and 4.57 mg/g of nickel from leaves. However, the range for L2 samples was wider, thus the team cannot definitively infer whether L2 samples produced more nickel than L3 samples. Also, from these samples it appears that leaves produce more nickel than stems with an average of 6.27 mg/g of nickel originating from the leaf compared to 1.65 mg/g originating from the stem. However, the sample, 1-L2, had more nickel production from the stem rather than the leaf, yet this can potentially be attributed to an idiosyncrasy in the data, leading to a recommendation of more trials being conducted. As will be discussed in the Alyssum and comparison sections, overall, more nickel was able to be extracted from leaf samples than stems, with 1-L2 being the only sample with the higher nickel production originating from the stem.

Using the total nickel concentrations from each sample, a yield percentage could be determined utilizing the standard values of nickel production for each plant species provided by a PhD student in the Sols & Eux laboratory. Below is a chart depicting the yield percentages of nickel for the Leptoplax samples.

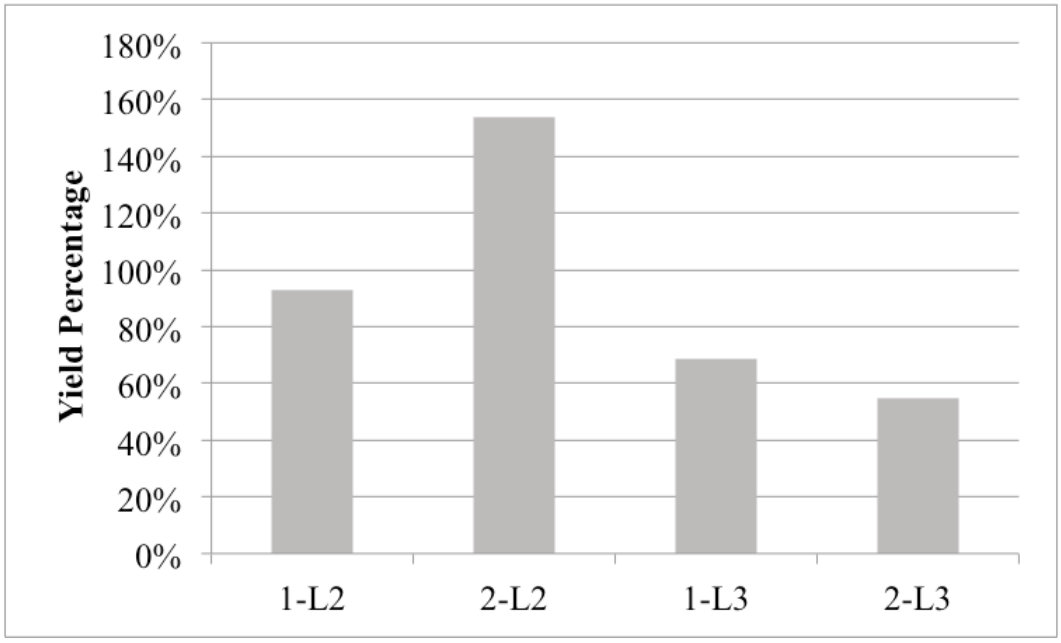

Figure 14: Nickel Yields in Leptoplax Samples

The L2 samples yielded more nickel based on the maximum nickel content that could be extracted with an average of 123% while the average yield for L3 was 62%. However, the reliability of this information must be questioned due to the fact that the team analyzed the plant species based on plant components and the standard values to analyze the yield was based on the whole plant. However, the team can infer that nickel is being extracted via the hydrometallurgical process, and an economic analysis may be conducted to evaluate the economic feasibility of this method.

4.2.2.2. Alyssum murale Samples

The Alyssum samples, 1-CC+, 2-CC+, 1-CC, and 2-CC+, were first analyzed separately from the Leptoplax samples to determine the difference between the Alyssum samples, themselves. Below is a chart detailing the overall concentrations of nickel in the plant samples.

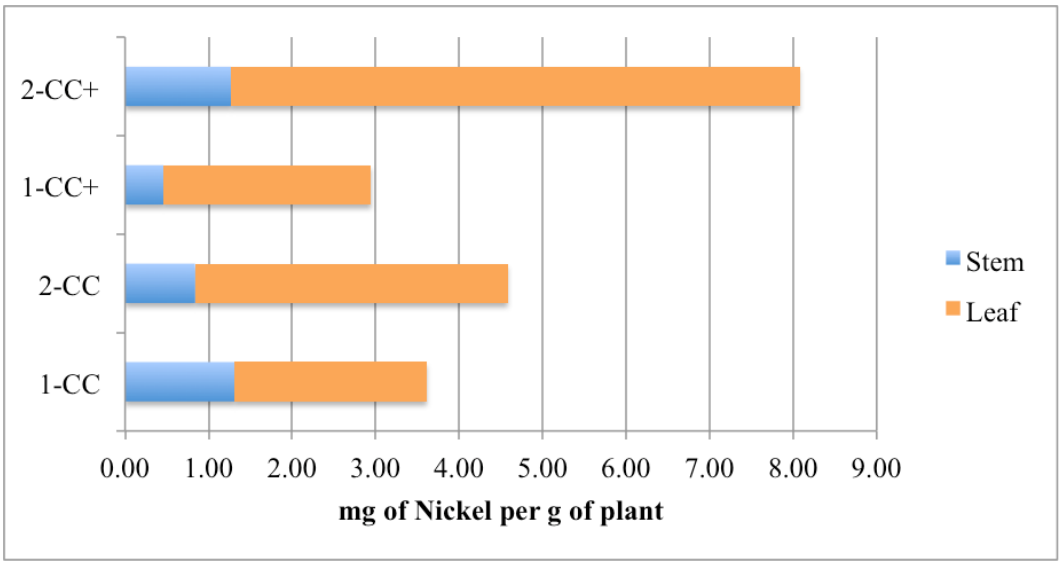

Figure 15: Chart of Nickel Concentrations in Alyssum Samples

The CC samples produced 1.32 mg/g and 0.84 mg/g of nickel from stems and 2.30 mg/g and 3.75 mg/g of nickel from leaves compared to the 0.46 mg/g and 1.27 mg/g of nickel from stems and 2.49 mg/g and 6.81 mg/g of nickel from leaves for the CC+ samples. On average, the CC+ samples produce more nickel than the CC samples; however, the range for the CC+ samples are wide compared to the CC samples being closer together. More trials would have to be conducted to accurately conclude which sample produces more Nickel. From this data, it can be inferred that the CC samples are more reliable due to the two samples having a smaller deviation. All the Alyssum samples have the most nickel production originating from the leaves. This is consistent with the predications made by the team.

Using the total nickel concentrations from each sample, a yield percentage could be determined utilizing the standard values of nickel production for each plant species. Below is a chart depicting the yield percentages of nickel for the Alyssum samples.

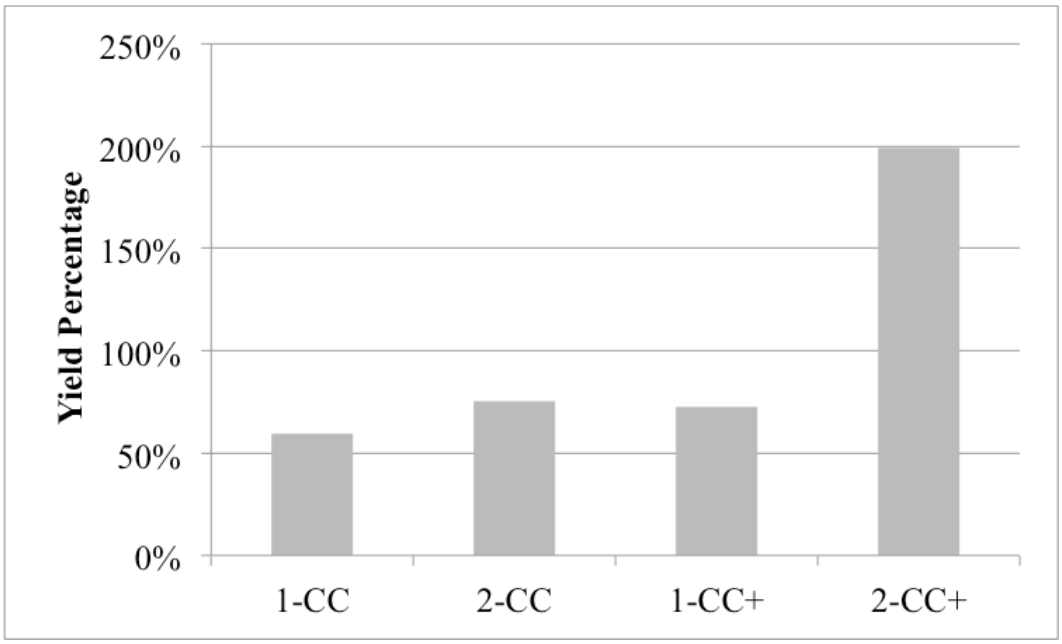

Figure 16: Chart of Nickel Yields in Alyssum Samples

On average, the CC+ samples yielded more nickel based on the maximum nickel content that could be extracted with an average of 136% while the average yield for CC was 67%. However, the reliability of this information must be questioned due to the fact that the team analyzed the plant species based on plant components and the standard values to analyze the yield was based on the whole plant. However, the team can infer that nickel is being extracted via the hydrometallurgical process, and an economic analysis may be conducted to evaluate the economic feasibility of this method.

4.2.2.3. Leptoplax emarginata and Alyssum murale Sample Analysis

After the separate analysis of each plant species, a comparison of the two was conducted to examine the differences in nickel concentrations and yield for Leptoplax and Alyssum. All samples were examined individually (e.g. 1-CC, 2-CC, 1-L2, 2-L2, etc.), and then each sample was averaged with its respective group (CC, CC+, L2, and L3).

Below are charts for the individual analysis of each sample for nickel concentration and nickel yield.

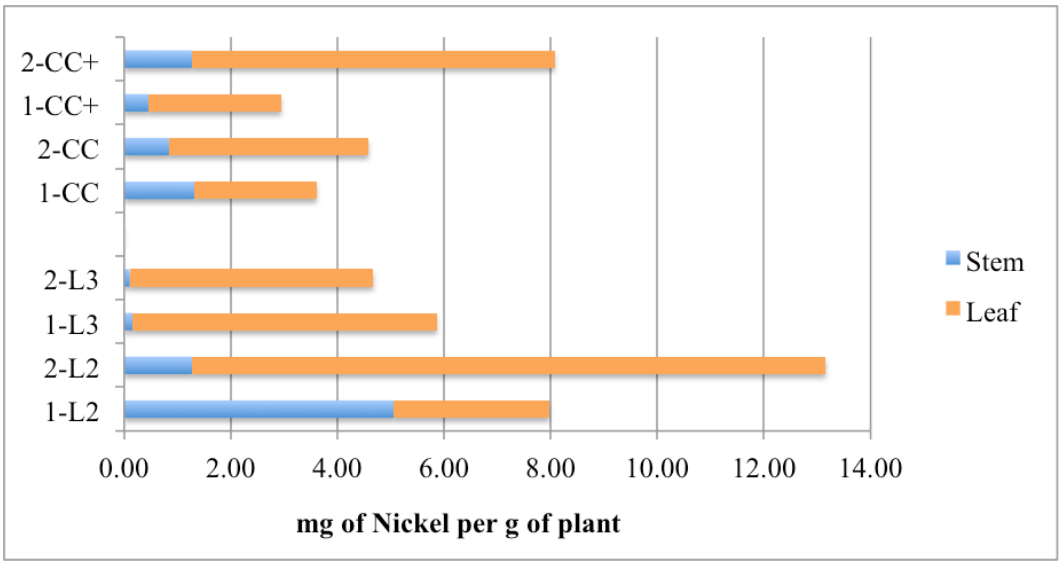

Figure 17: Chart of Nickel Concentrations in All Samples

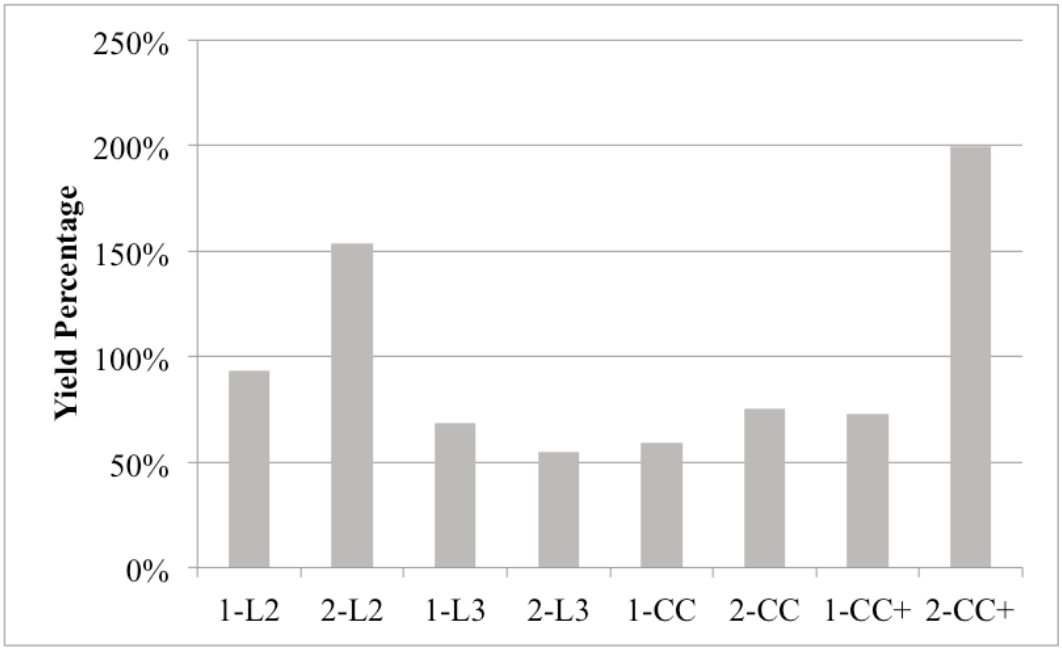

Figure 18: Chart of Nickel Yields in All Samples

All the samples, with the exception of 1-L2, had more nickel production from the leaves than the stems. The team hypothesized that the leaves would have more nickel production from the leaves due to leaves having a higher concentration of organic material. The team and the Sols & Eux laboratory believe that the free nickel is complexing with the organic material. However, this hypothesis will be analyzed later in *Fluorophore and Nickel Concentration Correlation*, by assessing the relationship between the fluorophores that indicate organic matter and the nickel concentrations in this section.

From the samples the team collected, the Leptoplax samples generally produce more nickel (mg) per the gram of plant biomass. Only the 2-CC+ sample for Alyssum produces more nickel overall compared to the Leptoplax samples. The team cannot infer anything from these results due to a small sample size; however, the team recommends further trials to be run to gain a better understanding of the concentrations produced from the Leptoplax and Alyssum samples.

All Alyssum and Leptoplax samples have yields over 50%, which from an early scientific standpoint is moderately fair. However, if a scale-up of this procedure were to be performed a more favorable yield would be preferable for economic success. The 2-L2 and 2-CC+ samples both have yields over 100%. As discussed previously, this may be due to the team analyzing the plants by components (leaf and stem) and the yield calculations require to look at the plant as a whole.

Below are charts of the averaged values for concentration and yield for the Leptoplax (L2 and L3) and Alyssum (CC and CC+) samples.

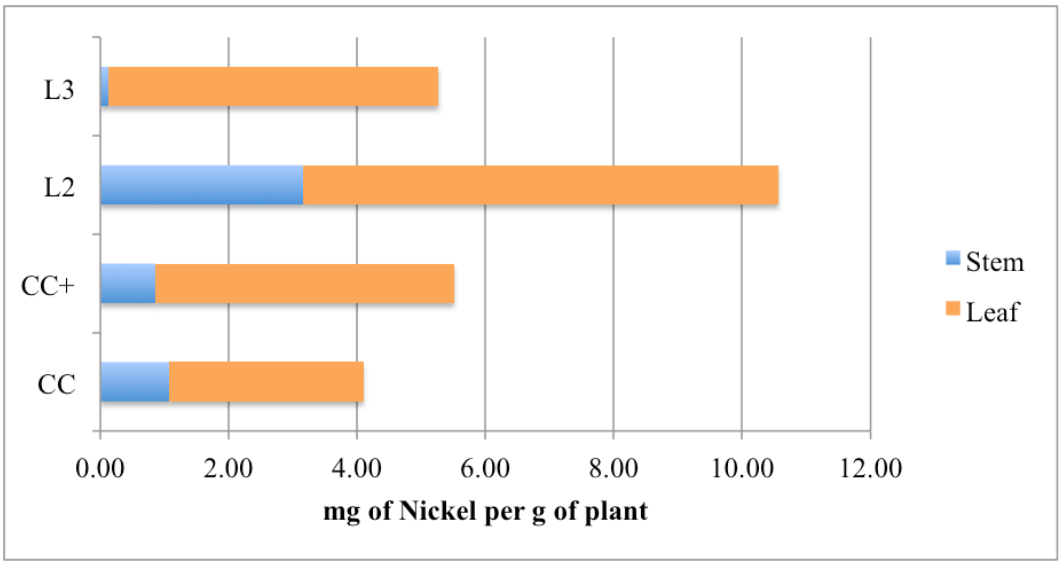

Figure 19: Chart of Averaged Nickel Concentrations

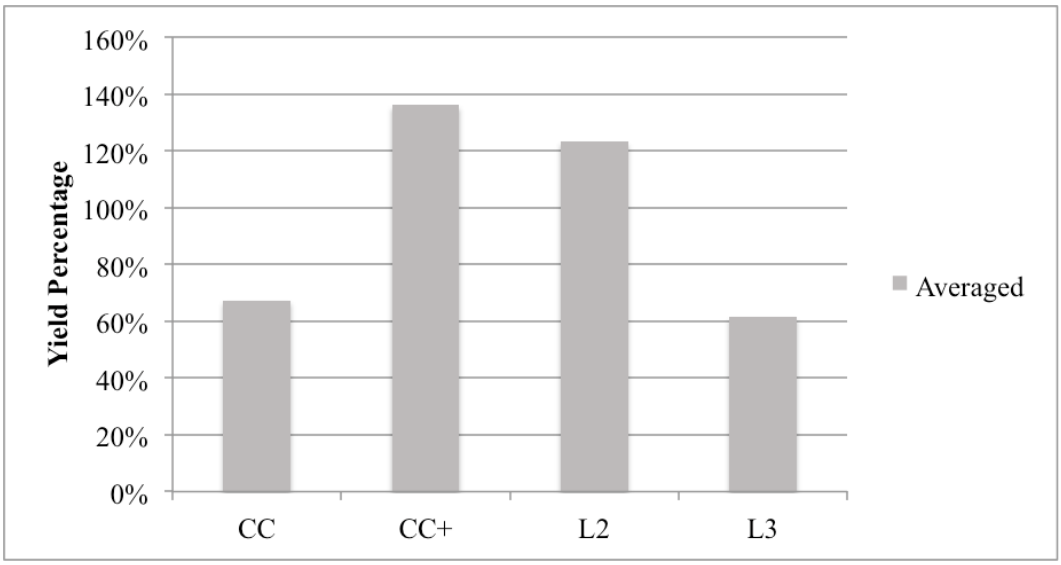

Figure 20: Chart of Averaged Nickel Yields

From the averaged values, a more distinct difference between the two species could be observed. From the concentration data, the L2 sample from the Leptoplax species has the greatest nickel production with over 10 mg/g being produced. The Leptoplax samples produce more nickel; however, the range between L2 and L3 is greater than the range between CC and CC+. The team cannot infer any conclusions from this, but suggest to the Sols & Eux laboratory to perform more trials.

From the average yield data, there is no difference between Alyssum and Leptoplax with both species having one sample with an average yield over 120% and one sample with a yield over 60%. As discussed previously, the team analyzing the plants by components (leaf and stem) and the yield calculations require to look at the plant as a whole thus the team recommends the Sols & Eux laboratory to separate the plants by component and develop a standard for nickel production from the components.

4.2.2.4. Affinity of Fluorophores to Complex with Nickel

After the analysis of the fluorophore dissolution program results to identify the fluorophores and nickel concentrations, the team hypothesized that potentially the higher intensity that a fluorophore presented that more nickel would be produced. The team hypothesized this due to a higher fluorophore intensity being indicative of more organic material, and it is believed that the free nickel is complexing with the organic material thus preventing it free nickel to be recovered via a hydrometallurgical process. The team tested this potential correlation by graphing the intensities of each fluorophore for each specific plant component, leaf and stem, versus the nickel concentration produced from that specific plant component.

Below are the charts for fluorophore 1 for the leaf and stem components.

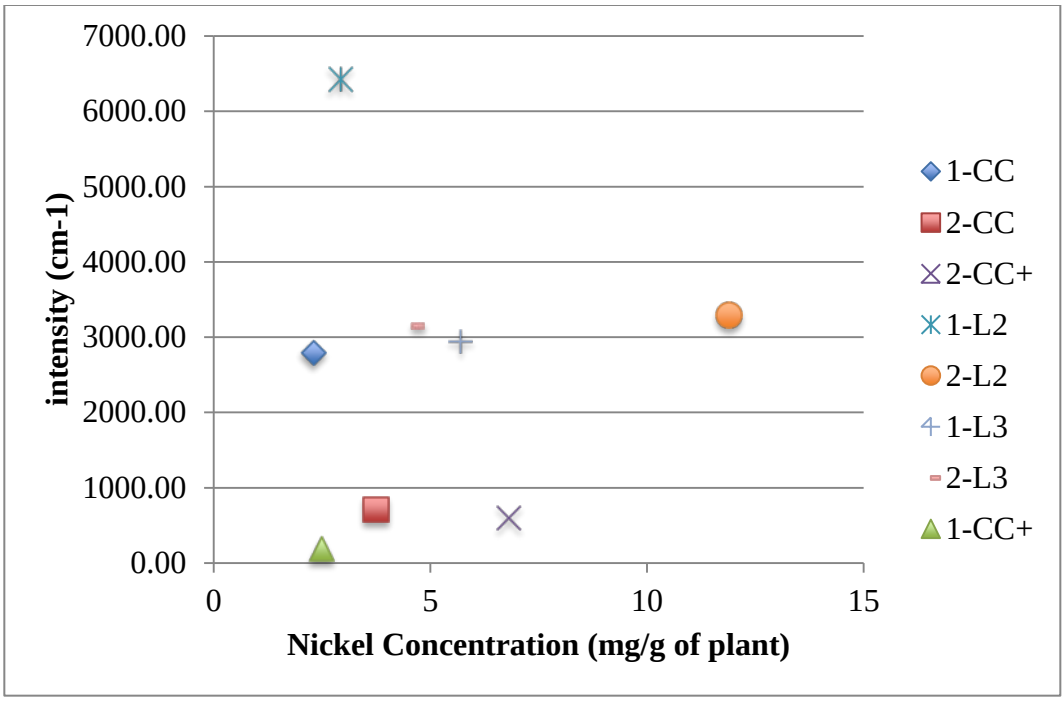

Figure 21: Chart of Leaf Correlation between Fluorophore 1 and Nickel Concentration

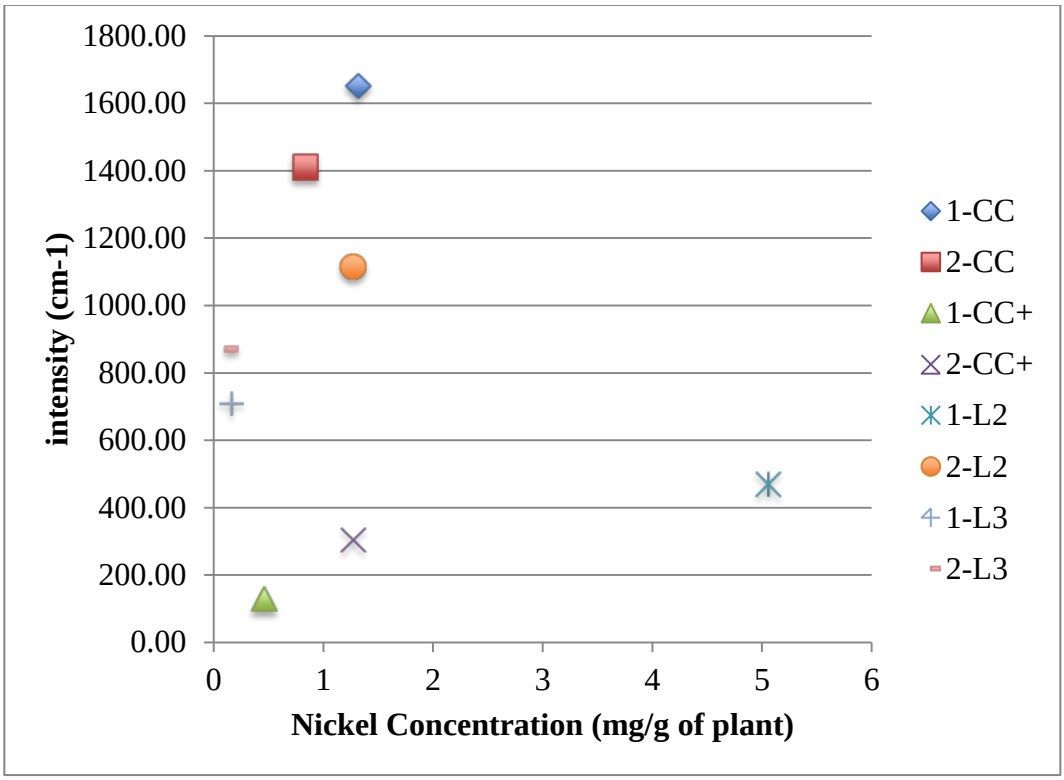

Figure 22: Chart of Stem Correlation between Fluorophore 1 and Nickel Concentration

When plotting the intensities versus the nickel concentrations for both stem and leaf for fluorophore 1, the team could not detect any correlation between the two. The points have no definitive association with each other thus the team could not report a relationship between the

two for fluorophore 1. The team hypothesized that fluorophore 1 would have the greatest chance of having a correlation between the intensities and nickel concentration due to it being an indicator of a variation of an indole compound, which is attached to the side chain of the organic compound glucosinolate. However, the team evaluated the same relationship for fluorophore 3 and 4 to determine whether this was just the case for fluorophore 1 or whether it is an issue for all three fluorophores.

Below are the charts for fluorophore 3 and 4 for the leaf and stem components.

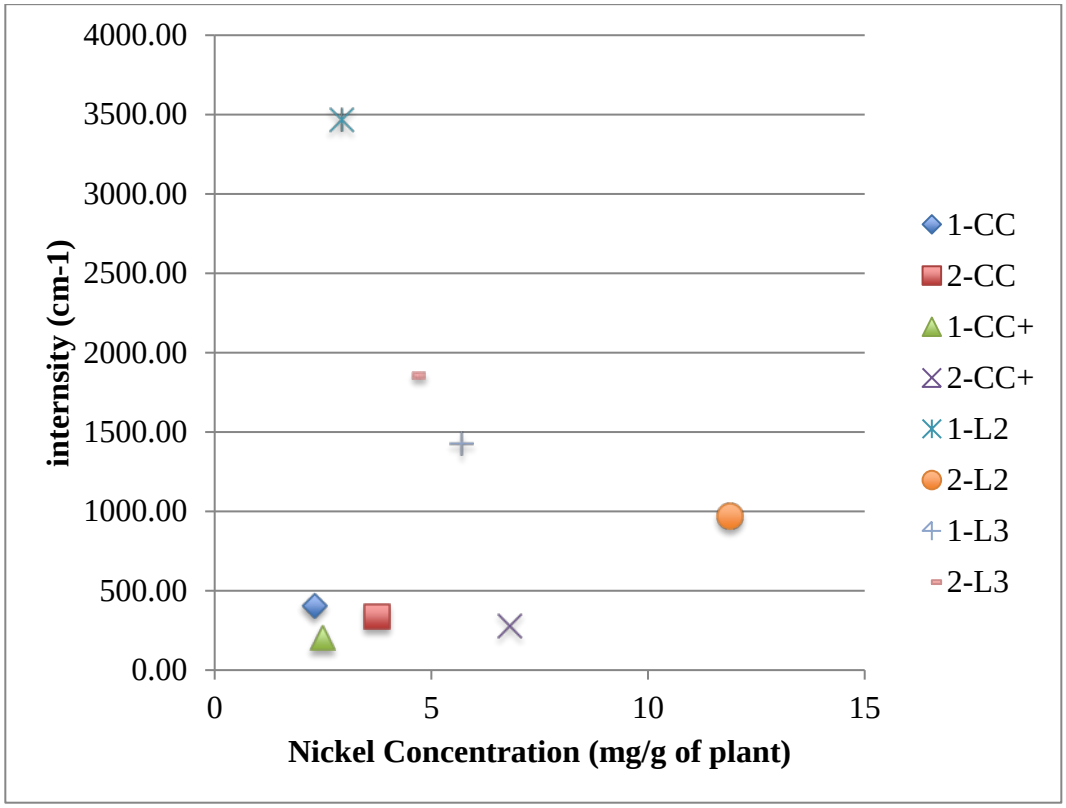

Figure 23: Chart of Leaf Correlation between Fluorophore 3 and Nickel Concentration

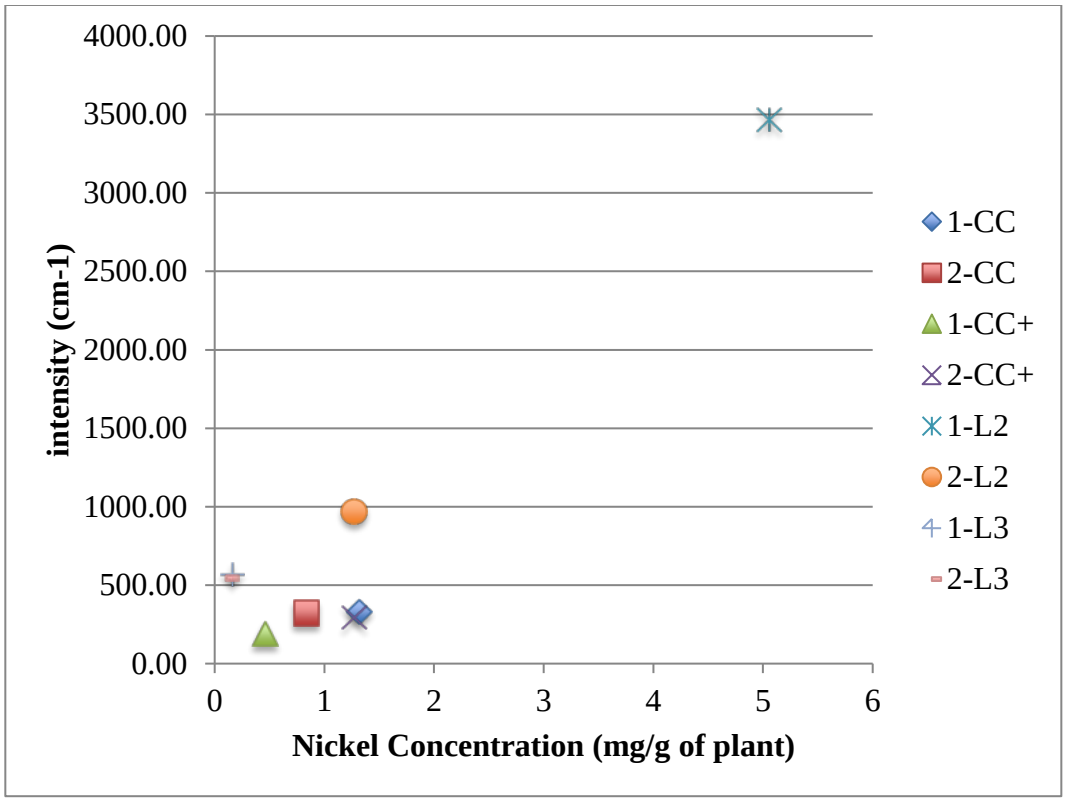

Figure 24: Chart of Stem Correlation between Fluorophore 3 and Nickel Concentration

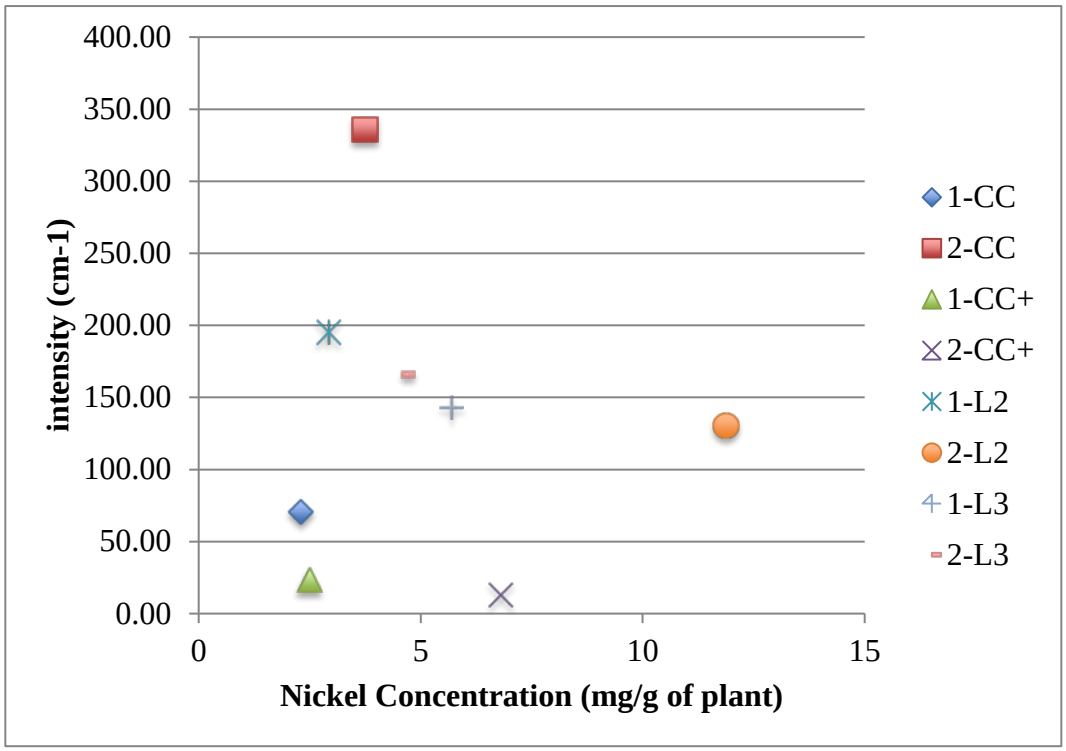

Figure 25: Chart of Leaf Correlation between Fluorophore 4 and Nickel Concentration

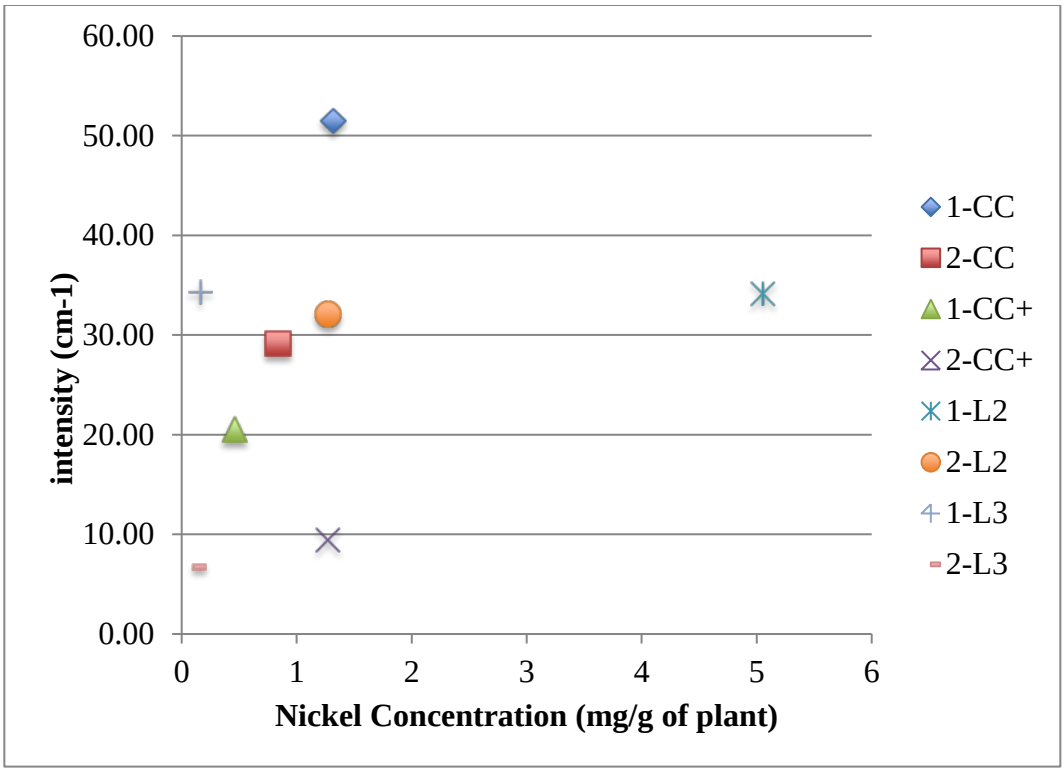

Figure 26: Chart of Stem Correlation between Fluorophore 4 and Nickel Concentration

Similar to fluorophore 1, the fluorophore 3 and 4 did not demonstrate any correlation between the intensities and nickel concentrations. All the points are scattered and no trendline can be seen for the leaf or stem samples. This indicates that the higher presence of organic material may not necessarily mean that the nickel is complexed with it. However, due to the small sample size, the team cannot infer any final conclusions thus the team recommends that further trials be run to fully analyze the correlation between the two.

4.2.3. Determination of Nitrogen and Total Organic Carbon (TOC) in Solution

After the team received the results from the TOC and nitrogen analysis, the team was able to determine the impact of the calculated nitrogen and TOC concentrations. Raw data obtained from the TOC and nitrogen test may be seen in Appendix E.1. As stated in the **Methodology** section, the team's samples were diluted by a factor of 10, but due to the high concentration of nitrogen in the 2-CC+ samples, the team ran a second trial. The new trial was run with newly prepared extract samples and with a dilution factor of 50. The samples tested with a dilution of 50 were 3-L2 Whole, 3-L3 Whole, 3-CC Whole, 3-CC+ Stem/Leaf, and 4-CC+ Whole. It must be noted that the team did not analyze the whole plant samples, only 3-CC+ Stem/Leaf, because the team could not ensure that the whole plant samples were an accurate representation of the plant compositions.

As previously described in the **Methodology** section, there were two different cultivation styles practiced when growing Alyssum. The styles being that CC+ would have fertilizer applied to the soil and CC would be grown simultaneously with nitrogen fixing legumes. Both practices were

implemented to provide nitrogen to the Alyssum plants. When cultivating Leptoplax, no forms of nitrogen supplements were utilized. With this knowledge, the team expected that the CC+ samples would have higher nitrogen concentrations than all other samples. After analysis, the team did observe this relationship between CC+ and other samples. The results of the nitrogen analysis for the samples may be seen in the figure below (the result of 3-CC+ were corrected for the difference in dilution).

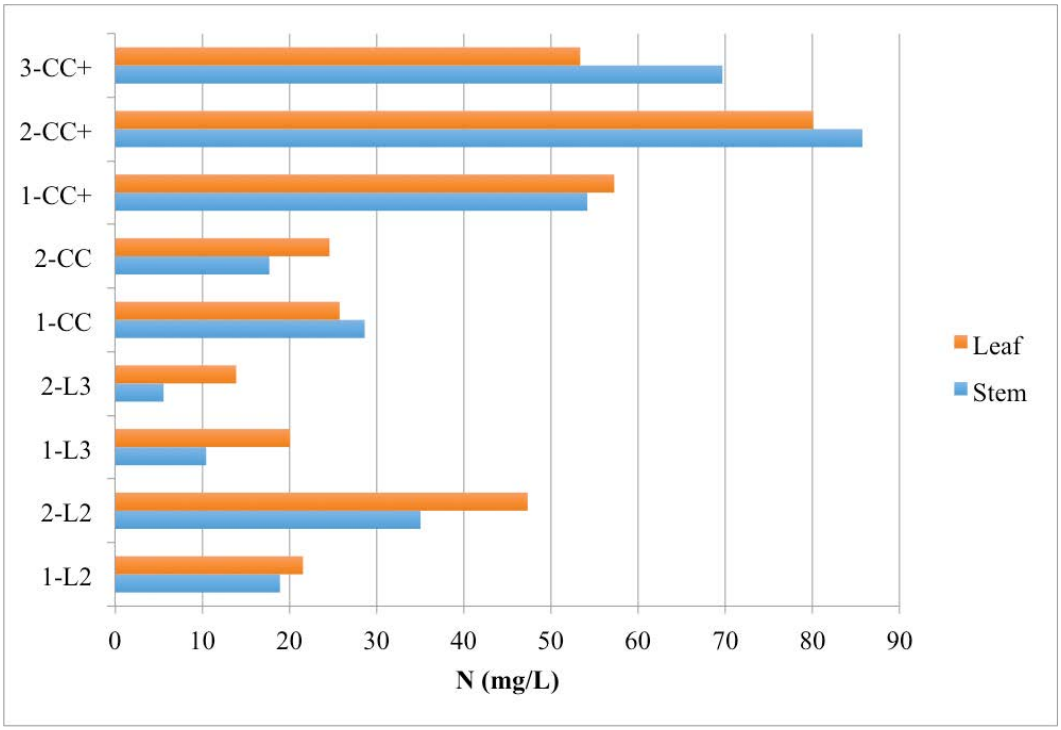

Figure 27: Nitrogen Concentration for Plant Components

Although, the results of the nitrogen analysis for 2-CC+ fell out of the set detectable range of 70 mg/L, the team may still compare the results of the 2-CC+ samples to the rest, but the team cannot ensure the precision of the results, solely that they are above 70 mg/L. Comparisons between CC+ and CC revealed that the use of fertilizer to increase nitrogen uptake increased the nitrogen within Alyssum by a factor of 6.4 within the leaves and a factor of 8.6 within the stems. Due to CC samples being grown simultaneously with legumes, the team also expected greater nitrogen concentrations when compared to Leptoplax. Nitrogen concentrations in L3 stems were lower than the concentrations in CC stems; however, when comparing the CC stem concentrations to L2 samples, this was not the relationship that was found. L2 and CC stem samples alternated in nitrogen concentration, preventing any definitive conclusions to be drawn on the impact of cultivating hyperaccumulators with legumes.

In addition to nitrogen analysis, the team performed an organic carbon analysis of the samples. The organic carbon results may be compared with the HIX values and fluorophore results in order to provide further insight into the organic compound concentrations within a hyperaccumulator. The results of the total organic carbon analysis may be seen in the figure below (3-CC+ samples were corrected for the difference in dilution factor).

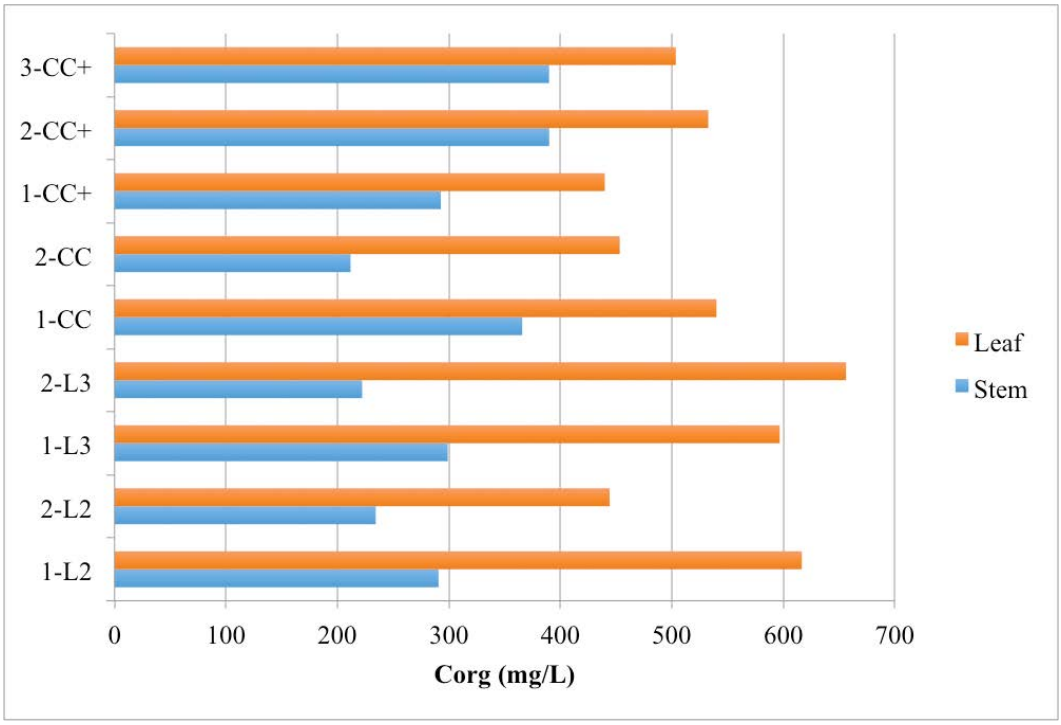

Figure 28: Total Organic Carbon Concentrations for Plant Components

Total organic carbon is consistently more concentrated in the leaves of hyperaccumulators than the stems. This corresponds with the higher HIX values in hyperaccumulator leaves than the stems, which is indicative of an increase in the C/H ratio and aromatic complexity. With this relationship the team can be confident of the reliability of the HIX results and the conclusions drawn from the index in regards to comparing the aromatic complexity of leaves versus stems.

In addition, the team compared these results to the fluorophores identified in the synchronous-50 fluorescence spectroscopy test. For all fluorophores, it is noted that Leptoplax leaves were significantly more concentrated than the stems. The fluorophores represent organic compounds and when reviewing the total organic carbon results for Leptoplax, the difference in concentration between the stems and leaves is apparent and corresponds to the fluorophore results. The relationship between fluorophore concentrations in Alyssum stems and leaves varied for each fluorophore, with the general trend being that Alyssum leaves are slightly more concentrated in the fluorophores than stems. This is represented in the total organic carbon results, as Alyssum leaves are more concentrated in total organic carbon than the stems; however, the two components are more similar in concentration than Leptoplax samples.

With this information, the team can confirm the greater concentration of organic compounds and more complex aromatics, such as glucosinolates and indole in the form of glucobrassicin, in hyperaccumulator plants. More specifically, there is a greater difference among Leptoplax components than Alyssum components.

5. Conclusions and Recommendations

Based on the methodology, results, and discussion, the team has several recommendations for the Sols & Eux laboratory. The recommendations will be based on the two phases, the *Sample Preparation* and *Sample Analysis*.

The recommendations for the *Sample Preparation* phase are as following:

1. Develop a more consistent method to grind the biomass.
2. Develop a more consistent method to extract the biomass during vacuum filtration.
3. Develop a method to analyze the amount of biomass that is extracted into the solution.
4. Run multiple of the same experiments to have a larger sample size.

During the grinding portion of the procedure, the stems and leaves were ground to different consistencies. The stems were more finely ground thus leading to smaller overall particles, while the leaves were unable to be finely ground by the blades due to their original smaller size. Thus, this led to different consistencies, which may have affected the solution while mixing and/or the extraction of the biomass during vacuum filtration. By developing a more consistent method, this will allow for fewer variables in the procedure.

During the vacuum filtration portion of the procedure, two different types of filters were used. A paper filter and a cloth filter were used to extract the liquid from the solution. These two different types of filters were used because the difficulties involving the vacuum filtration device. Initially, the team used a paper filter and some runs were successful, but during some runs, the liquid solution would not be filtered out and extracted. After several attempts to extract the solution, the team switched to a cloth filter which led to an overall better filtration; however, liquid would still remain in the biomass. The team recommends developing a consistent way to extract the liquid from the mixed biomass and water solution to reduce the amount of liquid that remains in the biomass.

The technique to analyze the amount of biomass extracted into the solution was very rudimentary. The team used the initial mass of the plant matter prior to filtration and the mass of the plant matter post filtration (and post drying) to determine the mass of the plant that was extracted into the solution. However, this method neglects the plant matter lost during the entire process. Material was lost during the collection of the material into the dish due to it adhering to the sides of the beakers. The team recommends that an experiment be conducted to determine the precise amount of plant material extracted which will help provide a more accurate value on how much biomass is extracted.

The last recommendations for the sample preparation phase would be to run more trials thus preparing more samples of the extraction with stems and leaves separated. This would increase the sample size and allow for more accurate analyses to take place thus providing Sols & Eux with more definitive answers.

Analysis of results obtained from the *Sample Analysis* lead the team to develop the following conclusions:

1. A form of indole and the aromatic compound glucosinolate are present in both species of hyperaccumulators.

2. There are two, possibly three, different organic compounds present in both species of hyperaccumulators aside from indole and glucosinolate.
3. Leaves of hyperaccumulators are more concentrated in total organic carbon and several organic compounds than their respective stems.
4. Fertilizer significantly impacted the nitrogen uptake of Alyssum *murale*, more so than simultaneous cultivation with nitrogen fixing legumes.

The peak that fluoresces with an emission wavelength of 280 nm signals the presence of an indole group attached to the aromatic compound glucosinolate to form glucobrassicin. This compound was present in both Leptoplax *emarginata* and Alyssum *murale.* Analysis of the fluorescence results revealed that the Leptoplax leaves are highly concentrated in indole when compared to their respective stems. This relationship was observed in Alyssum as well, but the difference in concentration between leaf and stem concentration was not large. Within the plant extracts of both species, there are two to three additional organic compounds. There is a possibility that fluorophore 2 represents glucosinolate, but the team did not observe the same trends when analyzing fluorophore 2 as seen with fluorophore 1. Fluorophores 3 and 4 are also indicative of organic compounds, which must go through further testing to be identified.

Corresponding with the fact that many of the fluorophores are more concentrated in the leaves, the team noted that there was a higher concentration of total organic carbon with the leaves of both plants. This is supported by the HIX values being higher in the leaves than the stems, symbolizing a greater C/H ratio in the leaves. Although, the team did observe the presence of various organic compounds and a greater aromatic complexity in the leaves, the team was not able to determine whether the presence of these compounds had an impact on nickel yield. When performing the analysis, a correlation could not be inferred; however, this may be due to the small sample size.

Finally, comparisons of the cultivation methods allowed the team to determine that fertilizer provided more readily available nitrogen than the nitrogen fixing legumes.

The recommendations for the *Sample Analysis* phase are as following:

1. Conduct research in order to identify fluorophores 2, 3 and 4.
2. Run multiple fluorescence spectroscopy tests and ICP-AES test to determine whether is a correlation between the detected fluorophores and nickel yield.
3. Run the multiple of the standard ICP-AES tests with the separate plant components (stem/leaves).

The identity of the fluorophores 2, 3 and 4 have not been confirmed, although there is a belief that fluorophore 2 is a component of glucosinolate. In addition, fluorophores 3 and 4 would be indicative of humic-like substances in natural water samples, but further research must be conducted to correctly identify the compounds. These compounds may be identified by comparing the disparities between concentrations in the stems and leaves of the species and to known compounds within the hyperaccumulators. Although, the experiments should also be run additional times with samples obtained from the serpentine plots prior to this research. When analyzing the fluorophore concentrations, the team did observe inconsistencies and the sample size was too small in order to make concrete conclusions on the fluorophore concentrations.

The experiments should be run multiple times in order to determine whether there is a correlation between nickel yield and fluorophore concentrations. The analysis revealed no clear correlation between any of the detected fluorophores and the nickel yield within the solution. It is known that there are organometallic complexes, which formed between organic compounds, which originate from the plants and nickel. Therefore, the experiments should be repeated in order to provide more data and determine which of the identified compounds may be responsible for the organometallic complexes.

As discussed in the *ICP-AES Mineral Analysis* section, the nickel yield percentages were calculated using a standard nickel value for the whole plant. However, the team separated the plant into separate components and tested those components and not the plant as a whole thus this account for some samples having a yield greater than 100%. Thus, the team recommends that the Sols & Eux laboratory test several separated plant components for their mineral concentrations to determine the standard value for the leaves and stems. This would give more precise data regarding the potential yield from the sample.

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Appendix A

Appendix A.1: Leptoplax *emarginata* Collection

	1 - L2		
Time	10:55	14:20	10:00 (18/01)
Dish (g)	65.5969		
Dish + Flower (g)	66.3296	66.2704	N/A
Flower (g)	0.7327	0.6735	**N/A**
Dish (g)	62.1832		
Dish + Leaf (g)	66.49	66.17	66.16
Leaf (g)	4.3068	3.9868	**3.9768**
Dish (g)	158.19		
Dish + Stem (g)	186.25	184.67	184.88
Stem (g)	28.06	26.48	**26.69**

	2 - L2		
Time	11:05	14:20	10:00 (18/01)
Dish (g)	35.1441		
Dish + Flower (g)	35.307	35.2856	N/A
Flower (g)	0.1629	0.1415	**N/A**
Dish (g)	48.13		
Dish + Leaf (g)	49.93	49.81	49.79
Leaf (g)	1.8	1.68	**1.66**
Dish (g)	131.9331		
Dish + Stem (g)	145.0156	144.04	143.86
Stem (g)	13.0825	12.1069	**11.9269**

	1 - L3		
Time	11:45	14:20	10:00 (18/01)
Dish (g)	12.13		
Dish + Flower (g)	12.1581	12.114	N/A
Flower (g)	0.0281	-0.016	**N/A**
Dish (g)	36.85		
Dish + Leaf (g)	39.17	39.03	38.97
Leaf (g)	2.32	2.18	**2.12**
Dish (g)	136.93		
Dish + Stem (g)	173.74	171.42	171.12
Stem (g)	36.81	34.49	**34.19**
Dish (g)	46.3167		
Dish + Seed (g)	50.396	50.1278	50.03
Seed (g)	4.0793	3.8111	**3.7133**

	2 - L3		
Time	12:10	14:20	10:00 (18/01)
Dish (g)	11.7435		
Dish + Flower (g)	11.7473	11.74	N/A
Flower (g)	0.0038	-0.0035	**N/A**
Dish (g)	11.2		
Dish + Leaf (g)	12.3027	12.24	12.11
Leaf (g)	1.1027	1.04	**0.91**
Dish (g)	247.37		
Dish + Stem (g)	292.19	289.49	288.96
Stem (g)	44.82	42.12	**41.59**
Dish (g)	104.508		
Dish + Seed (g)	107.47	107.22	106.98
Seed (g)	2.962	2.712	**2.472**

Appendix A.2: Leptoplax *emarginata* Grinding

1 - L2					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
26.69	18.34	44.16	25.82	0.87	3.26%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
3.9768	18.53	22.43	3.9	0.0768	1.93%
Flower					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
N/A	18.52	19.2	0.68	#VALUE!	#VALUE!

2 - L2					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
11.9269	18.57	30.41	11.84	0.0869	0.73%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
1.66	18.34	19.96	1.62	0.04	2.41%
Flower					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
N/A	18.56	18.73	0.17	#VALUE!	#VALUE!

Comments
L3 P2 Flower sample unusable due to very few collected sample

1 - L3					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
34.19	18.45	51.99	33.54	0.65	1.90%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
2.12	18.4	20.42	2.02	0.1	4.72%
Flower					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
N/A	18.4	18.45	0.05	#VALUE!	#VALUE!
Seed					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
3.7133	18.57	22.18	3.61	0.1033	2.78%

2 - L3					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
41.59	18.5	59.61	41.11	0.48	1.15%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
0.91	18.62	19.2	0.58	0.33	36.26%
Flower					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
N/A	18.49	N/A	N/A	#VALUE!	#VALUE!
Seed					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
2.472	18.47	20.91	2.44	0.032	1.29%

Appendix A.3: Leptoplax *emarginata* Extraction

Extraction - Stem				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Beaker (g)	112.38	115.94	115.31	106.16
Stem Prior Filtration (g)	4.00	4.00	4.01	3.99
Dish (g)	36.901	65.5968	35.1457	46.24
Dish + Stem Matter (g)	50.2954	77.0634	44.7624	57.2495
Stem Post Filtration (g)	13.3944	11.4666	9.6167	11.0095
Volume of Solution (mL)	84	90	87	90

Extraction - Leaf				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Beaker (g)	112.5442	116.1094	115.4828	106.2809
Leaf Prior Filtration (g)	2.02	1.63	1.96	0.72
Water Added (mL)	48.5	39.1	47	17.3
Dish (g)	48.2122	62.2109	104.7617	12.132
Dish + Leaf Matter (g)	55.7449	67.804	112.93	14.595
Leaf Post Filtration (g)	7.5327	5.5931	8.1683	2.463
Volume of Solution (mL)	40	33	38	14

Appendix A.4: Leptoplax *emarginata* Analysis

Extraction - Stem				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Water Added (mL)	96	96	96	96
Solution Removed (mL)	84	90	87	90
Final Stem + Dish (g)	40.0794	68.7128	38.3043	49.5862
Final Mass of Stem (g)	3.1784	3.116	3.1586	3.3462

Extraction - Leaf				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Water Added (mL)	48.5	39.1	47	17.3
Solution Removed (mL)	40	33	38	14
Final Leaf + Dish (g)	49.4102	106.0217	63.1644	49.5862
Final Mass of Leaf (g)	0.9102	66.9217	16.1644	32.2862

Extraction - Stem				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Stem Prior Filtration (g)	4.00	4.00	4.01	3.99
Final Mass of Stem (g)	3.1784	3.116	3.1586	3.3462
Max Plant Extracted (%)	20.54%	22.10%	21.23%	16.14%

Extraction - Leaf				
	1 - L2	***2 - L2***	***1 - L3***	***2 - L3***
Stem Prior Filtration (g)	2.02	1.63	1.96	0.72
Final Mass of Leaf (g)	1.198	0.9535	1.26	0.5136
Max Plant Extracted (%)	40.69%	41.50%	35.71%	28.67%

Appendix B

Appendix B.1: Alyssum *murale* Collection

1 - CC +		
Time	11:00	
Dish (g)	62.1686	
Dish + Leaf (g)	67.1	66.71
Leaf (g)	4.9314	4.5414
Dish (g)	247.38	
Dish + Stem (g)	269.18	267.67
Stem (g)	21.8	20.29

1 - CC		
Time	11:35	
Dish (g)	138.34	
Dish + Leaf (g)	144.68	144.1
Leaf (g)	6.34	5.76
Dish (g)	136.95	
Dish + Stem (g)	149.74	149.19
Stem (g)	12.79	12.24

2 - CC+		
Time	11:15	
Dish (g)	65.49	
Dish + Leaf (g)	70.47	70.195
Leaf (g)	4.98	4.705
Dish (g)	131.72	
Dish + Stem (g)	140.84	140.19
Stem (g)	9.12	8.47

2 - CC		
Time	11:45	
Dish (g)	104.53	
Dish + Leaf (g)	107.1	106.85
Leaf (g)	2.57	2.32
Dish (g)	158.18	
Dish + Stem (g)	175.29	174.08
Stem (g)	17.11	15.9

Alyssum Murale		
Time	11:50	
Dish (g)	144.44	
Dish + Material (g)	187.17	183.26
Leaf (g)	42.73	38.82

Appendix B.2: Alyssum *murale* Grinding

1 - CC+					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
20.29	18.72	38.83	20.11	0.18	0.89%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
4.5414	10.15	14.45	4.3	0.2414	5.32%

2 - CC+					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
8.47	18.59	27.05	8.46	0.01	0.12%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
4.705	10.14	14.65	4.51	0.195	4.14%

1 - CC					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
12.24	18.28	30.07	11.79	0.45	3.68%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
5.76	10.1	14.7	4.6	1.16	20.14%

2 - CC					
Stem					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
15.9	18.47	34.14	15.67	0.23	1.45%
Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
2.32	10.14	12.29	2.15	0.17	7.33%

Appendix B.3: Alyssum *murale* Extraction

Extraction - Stem					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	***Alyssum Murale 1***
Beaker (g)	103.7	115.29	105.09	106.11	115.32
Stem Prior Filtration (g)	4.00	4.02	4.00	4.00	4.01
Dish (g)	63.13	48.13	65.49	62.05	48.51
Dish + Stem Matter (g)	72.28	58.64	76.5	71.93	58.68
Stem Post Filtration Wet (g)	9.15	10.51	11.01	9.88	10.17
Stem Post Filtration Dry (g)	66	50.93	68.25	65.19	51.47
Volume of Solution (mL)	90	88	88	86	89

Extraction - Leaf					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	***Alyssum Murale 2***
Beaker (g)	107.6	105.03	115.94	106.11	115.93
Leaf Prior Filtration (g)	2.02	4.00	4.00	1.00	4.00
Water Added (mL)	48.5	96	96	24	96
Dish (g)	18.97	30.91	35.09	31.74	19.07
Dish + Leaf Matter (g)	24.58	44.09	46.33	35.36	29.15
Leaf Post Filtration wet (g)	5.61	13.18	11.24	3.62	10.08
Leaf Post Filtration dry (g)	20.09	33.29	37.51	32.24	22.05
Volume of Solution (mL)	47	85	80	22	91

Appendix B.4: Alyssum *murale* Analysis

Extraction - Stem					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	**Alyssum Murale 1**
Water Added (mL)	96	96	96	96	96
Solution Removed (mL)	90	88	88	86	89
Final Stem + Dish (g)	66	50.93	68.25	65.19	51.47
Final Mass of Stem (g)	2.87	2.8	2.76	3.14	2.96

Extraction - Stem					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	**Alyssum Murale 2**
Water Added (mL)	48.5	96	96	24	96
Solution Removed (mL)	47	85	80	22	91
Final Leaf + Dish (g)	20.09	33.29	37.51	32.24	22.05
Final Mass of Leaf (g)	1.12	2.38	2.42	0.5	2.98

Extraction - Stem					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	***Alyssum Murale 1***
Stem Prior Filtration (g)	4.00	4.02	4.00	4.00	4.01
Final Mass of Stem (g)	2.87	2.8	2.76	3.14	2.96
Max Plant Extracted (%)	28.25%	30.35%	31.00%	21.50%	26.18%

Extraction - Leaf					
	1 - CC +	***2 - CC +***	***1 - CC***	***2 - CC***	***Alyssum Murale 2***
Leaf Prior Filtration (g)	2.02	4.00	4.00	1.00	4.00
Final Mass of Leaf (g)	1.12	2.38	2.42	0.5	2.98
Max Plant Extracted (%)	44.55%	40.50%	39.50%	50.00%	25.50%

Appendix C

Appendix C.1: Rerun Plant Collection

3 - L2		
Time	13:23	
Dish (g)	132.92	
Dish + Plant (g)	169.52	166.78
Plant (g)	36.6	33.86

3 - L3		
Time	13:30	
Dish (g)	134.4	
Dish + Plant (g)	173.13	170.97
Plant (g)	38.73	36.57

4 - CC + Whole		
Time	13:33	
Dish (g)	246.96	
Dish + Plant (g)	302.54	298.18
Plant (g)	55.58	51.22

3 - CC Whole		
Time	13:38	
Dish (g)	210.4	
Dish + Plant (g)	256.33	252.84
Plant (g)	45.93	42.44

3 - CC + Stem		
Time	13:49	
Dish (g)	136.93	
Dish + Plant (g)	154.12	152.84
Plant (g)	17.19	15.91

3 - CC + Leaf		
Time	13:51	
Dish (g)	138.3	
Dish + Plant (g)	151.42	150.28
Plant (g)	13.12	11.98

Appendix C.2: Rerun Plant Grinding

3 - L2					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
33.86	18.41	44.82	26.41	7.45	22.00%
4 - CC + Whole					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
51.22	18.68	69.37	50.69	0.53	1.03%
3 - CC + Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)**	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
11.98	20.25	32.06	11.81	0.17	1.42%

3 - L3					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
36.57	18.62	52.31	33.69	2.88	7.88%
3 - CC Whole					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
42.44	18.52	60.69	42.17	0.27	0.64%
3 - CC + Leaf					
Mass Before Grind (g)	*Mass of Bottle (g)*	*Bottle + Ground Material (g)*	*Ground Material (g)*	*Loss (g)*	*Loss (%)*
15.91	10.08	25.93	15.85	0.06	0.38%

Appendix C.3: Rerun Plant Extraction

Extraction - Whole				
	3 - L2	***3 - L3***	***4 - CC + Whole***	***3 - CC Whole***
Beaker (g)	115.34	107.58	115.32	74.37
Prior Filtration (g)	4.01	4.01	4.01	4.04
Dish (g)	63.15	36.85	48.44	74.93
Dish + Matter (g)	73.71	47.88	58.63	90.18
Post Filtration Wet (g)	10.56	11.03	10.19	15.25
Post Filtration Dry (g)	66.37	39.69	51.23	77.49
Volume of Solution (mL)	90	87	90	81

Extraction - CC+		
	Stem	***Leaf***
Beaker (g)	115.93	105.86
Prior Filtration (g)	4.00	4.01
Water Added (mL)	96	96
Dish (g)	74.38	74.89
Dish + Matter (g)	87.47	83.68
Post Filtration wet (g)	13.09	8.79
Post Filtration dry (g)	77.16	77.29
Volume of Solution (mL)	81	91

Appendix C.4: Rerun Plant Analysis

Extraction - Whole				
	3 - L2	***3 - L3***	***4 - CC + Whole***	***3 - CC Whole***
Water Added (mL)	96	96	96	96
Solution Removed (mL)	90	87	90	81
Final Stem + Dish (g)	66.37	39.69	51.23	77.49
Final Mass of Stem (g)	3.22	2.84	2.79	2.56

Extraction - CC+		
	Stem	***Leaf***
Water Added (mL)	96	96
Solution Removed (mL)	81	91
Final Leaf + Dish (g)	77.16	77.29
Final Mass of Leaf (g)	2.78	2.4

Extraction - Whole				
	3 - L2	***3 - L3***	***4 - CC + Whole***	***3 - CC Whole***
Stem Prior Filtration (g)	4.01	4.01	4.01	4.04
Final Mass of Stem (g)	3.22	2.84	2.79	2.56
Max Plant Extracted (%)	19.70%	29.18%	30.42%	36.63%

Extraction - CC+		
	Stem	***Leaf***
Leaf Prior Filtration (g)	4.00	4.01
Final Mass of Leaf (g)	2.78	2.4
Max Plant Extracted (%)	30.50%	40.15%

Appendix D

Appendix D.1: Fluorescence Dissolution Program Results

	Fluoro 1	Lambda1	Width1	Fluoro 2	Lambda2	Width2	Fluoro 3	Lambda3	Width3	Fluro 4	Lambda4	Width4
1-L2 Leaf	6431.48	274.25	7.85	5901.82	286.91	11.76	3468.67	351.73	14.73	195.46	400.70	10.00
1-L2Stem	470.41	290.00	18.23	1214.74	280.00	10.71	359.85	350.67	17.34	34.15	401.02	24.81
2-L2 Leaf	3294.96	280.71	10.05	256.03	300.00	17.41	969.40	353.54	15.19	130.43	399.15	12.64
2-L2 Stem	1115.82	280.16	10.45	203.83	300.00	15.86	270.68	351.54	16.22	32.09	399.22	17.60
3-L2 Whole	753.32	276.23	8.85	533.02	292.15	13.43	492.91	353.25	15.33	41.00	402.22	10.97
1-L3 Leaf	2940.44	275.18	8.22	2226.70	288.68	12.20	1424.83	353.20	15.09	142.75	401.00	10.91
1-L3 Stem	709.23	290.00	17.88	1712.26	280.00	11.38	568.30	352.58	14.54	34.30	398.82	19.52
2-L3Leaf	3149.61	275.03	7.92	2602.23	288.69	11.98	1854.03	353.21	14.70	165.89	401.00	10.52
2-L3 Stem	869.76	274.63	8.35	856.72	290.57	13.19	544.04	351.95	14.75	6.74	401.80	90.21
3-L3 Whole	1869.81	279.12	9.94	586.96	297.56	14.62	603.41	353.04	17.52	64.18	403.58	16.01
1-CC Leaf	2802.10	276.81	8.92	408.77	295.19	14.16	406.27	348.82	20.34	70.91	399.57	18.53
1-CC Stem	1653.52	277.44	9.27	385.18	298.69	14.93	333.61	349.72	18.47	51.53	390.00	34.24
2-CC Leaf	711.68	287.69	2.39	3158.18	281.16	10.00	335.94	345.00	27.12	71.02	404.05	11.29
2-CC Stem	1411.05	277.33	9.48	388.57	294.75	13.89	322.72	351.30	16.14	29.14	401.67	11.92
3-CC Whole	2637.59	278.32	9.39	290.04	302.07	14.29	355.43	351.61	17.77	70.68	400.20	14.35
1-CC+ Leaf	193.09	287.49	2.00	1544.23	280.63	10.00	206.77	345.00	27.67	23.65	410.00	16.02
1-CC+ Stem	130.07	290.00	17.30	1026.90	280.71	10.00	193.61	346.22	24.40	20.56	410.00	14.86
2-CC+ Leaf	600.25	287.41	2.58	2691.81	280.85	10.00	282.11	346.07	30.37	12.95	390.00	100.00
2-CC+ Stem	303.75	287.57	2.13	1785.19	281.18	10.00	295.34	345.00	29.41	9.48	398.48	74.70
3-CC+ Leaf	2833.41	280.88	9.51	278.26	310.00	26.43	245.86	352.42	17.50	106.60	390.00	25.04
3-CC+ Stem	2478.78	281.06	10.11	261.19	310.00	10.00	482.57	348.87	23.23	101.29	403.30	14.47
4-CC+ Whole	1574.95	280.92	9.45	201.43	310.00	26.23	196.63	353.85	16.05	96.10	390.00	24.94
Average		281.28			292.26			350.39			399.81	
STDV		5.55			10.34			3.07			5.60	

Appendix E

Appendix E.1: TOC and Nitrogen Results

Name	N (mg/L)	Corg mg/L	C/N
1-L2 Leaf	21.56	616.5	28.59
1-L2 Stem	18.92	290.6	15.36
2-L2 Leaf	47.32	444.1	9.39
2-L2 Stem	35.04	234.3	6.69
3-L2 Whole	1.46	44.5	30.47
1-L3 Leaf	20.06	596.7	29.75
1-L3 Stem	10.46	298.9	28.58
2-L3 Leaf	13.90	656.2	47.21
2-L3 Stem	5.57	222.1	39.85
3-L3 Whole	6.46	68.7	10.64
1-CC Leaf	25.76	540.0	20.96
1-CC Stem	28.64	365.7	12.77
2-CC Leaf	24.60	453.2	18.42
2-CC Stem	17.70	211.7	11.96
3-CC Whole	5.46	75.5	13.83
1-CC+ Leaf	57.25	439.8	7.68
1-CC+ Stem	54.16	292.7	5.40
2-CC+ Leaf	80.07	532.6	6.65
2-CC+ Stem	85.70	389.9	4.55
3-CC+ Leaf	10.67	100.7	9.44
3-CC+ Stem	13.93	78.0	5.60
4-CC+ Whole	12.15	107.3	8.83

Attachment 1

Design Analysis

A decommissioned nickel mine located in Spain recently had an environmental assessment conducted to assess the soil quality. The mining company suspects that the area, which once held the metal refining building and tailing disposal site, has an elevated concentration of nickel within the soil. The assessment confirmed this suspicion and determined that the contaminated site spans over an area of 1 hectare, with an average nickel concentration of 0.6 g/kg of soil within the first half meter (0.5 m) of the profile. There are several proposed remediation options and the two that are being considered are as follows: agromining, with the end nickel product being ammonium nickel sulfate hexahydrate (ANSH), or soil removal with backfilling and a cap. The owners of the site are not looking to cap the contaminated soil as they wish to use it for agriculture within the next seven years. The owners have chosen to proceed with the agromining option, as soil removal is costly and the end product of the agromining process may be sold for further use in industry. In addition, testing has revealed that the contaminated soil has similar properties to ultramafic serpentine soil with a density of 3.0 g/cm^{-3}.

Plant Cultivation:

The remediation design will involve the hyperaccumulator Alyssum *murale*. This specific hyperaccumulator was chosen because it naturally grows in ultramafic soils and studies have demonstrated its significant ability to accumulate nickel. The Alyssum *murale* plants will be planted throughout the site at a concentration of 16 plants/m^2, resulting in 160,000 hyperaccumulators plants total. The plants will be allowed to grow for approximately 10 and will rely on rain as a source of water. To promote growth, phosphorous fertilizer was added at 100 kg/ha in the form of $Ca(H_2PO_4)_2 \cdot 2H_2O$ and nitrogen added at 150 kg/ha in the form of NH_4NO_3.In addition, herbicide will be added to inhibit weed growth. With the addition of these two products, the average dry plant mass be 60 g and the nickel accumulation rate is expected to be an average of 20 g/kg dry plant mass. This will result in 9,600 kg of plant mass and a nickel accumulation of approximately 192.0 kg. Once the plants have matured, they will be cut at the base of the stem and allowed to dry for one day on the site. The plants will then be collected and proceed to a plant or laboratory for the production of ANSH. The site will have to undergo this process 5 times over a 5 year time period (1 harvest per year) in order to lower the concentration to 0.54 g/kg of soil from the original 0.6 g/kg.

Ammonium Nickel Sulfate Hexahydrate:

The patented procedure to produce ANSH from Alyssum *murale* ashes will be implemented in the design (Barbaroux et al., 2012). However, modifications proposed by Zhang and the team will be used to reduce cost and optimize nickel extraction. This patented process involves four major steps: ashing, leaching, purification, and crystallization. The final crystal will contain 13.2% nickel and have a purity of 99%. A detailed diagram of the procedure, including modifications can be seen in the figure below.

In order to optimize the process and reduce cost, the following specifications will be followed:

Ashing: To prepare the plant material, the biomass will be finely ground. The ground plant material (25 g biomass) will then be incinerated in an oven at 550°C for 2 hours. The ashes will be washed twice with deionized water in order to remove highly soluble potassium. Washing will be completed with a 20% solids concentration and a rotational speed of 500 rpm for 15 minutes. The ashes will be allowed to dry prior to leaching.

Leaching: The acid leaching process will use 2 M sulfuric acid (H_2SO_4) will to leach the plant biomass and an ash solids concentration of 10% at 95°C for 2 hours. This will result in 99.5 ± 7.9% nickel transfer to the leachate from the plant biomass.

Purification: The leachate has many impurities that must be removed in order to produce a valuable crystal. The first purification process will require pH neutralization and Mg precipitation step to remove several impurities. The pH of the leachate will be raised to approximately 4.5 with a suspension of 10% of calcium hydroxide ($Ca(OH)_2$), as opposed to NaOH which was used in the patented procedure. This step is done in order to remove excess H_2SO_4 and Fe in the form of $CaSO_4$ and $Fe(OH)_3$, both will participate out. The neutralization agent was switched to $Ca(OH)_2$ so that cost are reduced and the production of Na_2SO_4 is eliminated. This step of the purification process will remove 95 ± 5.3% of the Fe in the leachate. The next step to the purification process is the precipitation of Mg. Magnesium will be removed by adding 10% more of the stoichiometric need of NaF to form the precipitate, MgF_2. The leachate will be filtered to remove all precipitates and 93.5 ± 3.3% of the nickel will be recovered.

Crystallization: The final step is crystallization with further purification. To crystalize the leachate and produce ANSH, a mass of ammonium sulfate ($(NH_4)_2SO_4$) will be added so that it is in 20% excess. The solution will then be heated to 60°C to allow the ammonium sulfate to dissolve and then brought down to room temperature. The solution will then be allowed to crystallize at 0°C for 4 hours and while being stir with a magnetic stir bar. This crystal will then be dissolved in deionized water at a concentration of 17 g/100 mL at 60°C. The solution will once again be brought down to 0°C and allowed to crystallize once more for 4 hours to form the final product, ANSH, with a purity of 99.1%. Overall, 18.4 kg of dry plant biomass will produce 1 kg of ANSH.

Overall, each harvest from the contaminated site can produce up approximately 522 kg of ANSH.

Cost Analysis

The affordability of growing the hyperaccumulator crops, as opposed to soil removal and capping, was the prime reason the owners chose the former remediation option. The cost of cultivating one hectare of land with a hyperaccumulator can range from 250-500 USD. The addition of fertilizer and herbicide to the field will cost approximately 150 USD/ha, the cost of cultivation is estimated to be $400/ha harvest with a crop value of $16,000/ha.

Cost of producing the ANSH product heavily depends on the selected chemicals to neutralize and leach the biomass. The patented procedure utilized NaOH to neutralize the pH of the leached biomass suspension; however, studies have shown that $Ca(OH)_2$ can also be used and will reduce the costs of producing ANSH. Although, this process has not been scaled up to a commercial size, studies have projected the following cost breakdown for a production plant or laboratory:

Table 1: ANSH Production Cost Breakdown

Category	Percentage (%)
Chemicals	10.8
O&M (Excluding Chemicals)	38.8
Indirect and General	42
Financing	20.4

The owner's payment will be divided into the above categories once received by the production plant/laboratory. Currently, there is no precise cost for ANSH production because the process has not been commercialized to include salaries of workers. Although, one estimate predicts that the chemical component will cost less than 10 USD/kg ANSH, making the overall production cost nearly 92 USD/kg ANSH.

Once the ANSH crystals have been formed, they may be sold to industry for approximately 600 USD/kg, provided that the purity is above 99%. Each harvest can yield a profit of 313,000 USD and a net profit of 265,000, approximately.

Work Cited

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Attachment 2

Motive for Obtaining Professional Engineering License

There are several steps one must take in order to become professionally licensed engineer, the first begins upon graduation when one can take the Fundamentals of Engineering exam in their respective discipline. Passing this exam allows the person to officially become an “Engineer In Training” and after 4 years in industry, one can take the Professional Engineering exam and officially call themselves an engineer. Becoming a certified professional engineer (P.E.) can open many doors for an engineer professionally and personally, and therefor should be a milestone goal for any aspiring engineer.

Professionally, obtaining a P.E. license enables an engineer to advance within their respective field. In certain engineering sectors, such as consulting or a private practice, becoming a professional engineer is necessary. Legally, professional engineers are the only people who may develop, sign, stamp and submit design drawings. Gaining the experience that is necessary to prepare design drawings and be the official P.E. on a portion of a project takes many years of practice, but without the official licensure, this will not be an option. In addition, within the consulting field, certain levels of promotion may only be reached with a professional license. The opportunities for professional development that a P.E. license provides also extend beyond being able to stamp design drawings. In order to start one’s own engineering firm, they must be a P.E. by law. In addition, when working in industries that involve potential lawsuits, such as fire protection, a certified engineer is the only professional who can testify in court to the merits of a fire protection design. A professional license sets an engineer apart from others and ensures a level of competence, making them valuable to a company and sought out by employers; without a P.E. professional advancement may be stunted. Although, there are industries that do not require an engineer to be on track to obtaining a license, those are becoming rarer because of the realized value of the P.E. license.

In addition to professional development, holding a P.E. license ensures a level ethical responsibility. By becoming a licensed engineer, one binds them self to the Code of Ethics set by the National Society of Professional Engineers, and although all engineers should always abide by this code, the P.E. license provides an additional sense of security to clients and employers. This aspect of being able to call oneself a professional engineer is arguably one of the most important. Engineering is considered to be a highly regarded profession, on a similar level to doctors and lawyers because of the responsibility each owes to their clients and the public. Failure to comply with the Code of Ethics is a serious offense and can lead to the license being revoked, and in some cases legal action will be taken against the engineer. With every professional engineer having an obligation to uphold the outlined ethical practices, they are highly sought after for employment and typically have a priority during company downsizing events.

On a personal level, holding a professional engineering license is also rewarding. This license is a measure of a person’s ability to complete quality work while carrying out ethical practices. It also provides others with a sense for a person’s drive and work ethic as those holding a P.E. are highly respected. Obtained a professional engineering license is milestone that many engineers should

strive towards because of the opportunities it provides for professional development and personal reward.