Phytoremediation of Vanadium and Nickel from Wastewater with *Acorus calamus*

by

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Abstract

Acorus calamus is an important medicinal plant in many cultures around the world. This plant's recorded history reaches as far back as 287 BCE, where its main uses were water purification and as a medicinal "cure-all". In part one, I examined the history of *A. calamus*, its applications in the modern world and propagation methods. The two main propagation methods I tested were via rhizomes and seeds. Rhizome growth trials were more successful in overall plant yield than the seeds. Rhizomes planted in peat had 57% plant yield, while the no-peat treatment had 40% plant yield. Part two of the study examined *A. calamus* ability and efficiency in extracting vanadium and nickel from experimentally treated waters. Vanadium and nickel were chosen for their importance in the environment and their enrichment in bitumen, specifically tailings ponds. Nickel is an important nutrient for plant survival and vanadium is not. *Acorus calamus* plants were grown in a hydroponic system with 188 plants grown inside a clean air growth chamber for three months. Every day the plants were treated with nickel-enriched solutions at three concentrations (0.0, 0.01, 0.10 mg/L) and vanadium (0.0, 0.025, 0.25 mg/L). At the end of three months, plant tissues were harvested and analyzed for metal concentrations. The results showed *A. calamus* extracted both vanadium and nickel from the contaminated waters with maximum values of 0.6 mg/kg and 16.3 mg/kg, respectively. Results indicate *A. calamus* would be an excellent candidate for phytoremediation at contamination levels well above what is found naturally occurring in Alberta's water sources.

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Glossary of Terms and Definitions

<u>*Acorus calamus*</u> – a perennial monocot plant that grows in wet soils or waters and regenerates by rhizome or seed dispersal.

<u>Bitumen</u> –a generic term applied to natural inflammable substances of variable color, hardness, and volatility, composed principally of a mixture of hydrocarbons substantially free from oxygenated bodies. Bitumen is sometimes associated with mineral matter, the nonmineral constituents being fusible and largely soluble in carbon disulfide, yielding water-insoluble sulfonation products. Petroleum, asphalts, natural mineral waxes, and asphaltites are all considered bitumen. (Jackson, Julia A., et al, 2011)

<u>Nickel</u> – a chemical element used to make stainless steels, nickel is found in the earth's crust, the average abundance in the earth's Upper Continental Crust is 75 ppm (Taylor, 1964). Nickel is the 24th most abundant element in the earth's crust, and the 5th most abundant element in the earth's biosphere (Chau and Kulikovsky-Cordeiro, 1995).

<u>Phytoextraction</u> – a subprocess of phytoremediation, explicitly using plants to remove undesirable metals and contaminants from soil or water (Powter, 2002).

<u>Phytoremediation</u> - the use of living plants for *in situ* removal of contaminants found in soils, surface water, and groundwater (Powter, 2002).

<u>Propagation</u> – the growth and spread of plants by natural or human-assisted processes.

<u>Remediation</u> – the removal, reduction, or neutralization of substances, wastes or hazardous material from a site so as to prevent or minimze any adverse effects on the environment now or in the future (Powter, 2002).

<u>Reclamation</u> – the process of converting disturbed land to its former or other productive uses (Powter, 2002).

<u>Restoration</u> – the process of restoring site conditions as they were before the land disturbance (Powter, 2002).

<u>Stratification</u> – the breaking of seed dormancy by exposing the seed to prolonged or repeated freezing under moist conditions. However, alternating warm and cold stratification methods are also frequently used. These methods are used to improve germination frequency (Powter, 2002).

<u>Vanadium</u> – a transition metal used to make alloy steels and found naturally occurring in the earths crust. Average abundance in the earth's Upper Continental Crust is 135 ppm (Huang et al., 2015).

<u>Inductively coupled plasma mass spectrometry (ICP-MS)</u> – a type of mass spectrometry that uses an inductively coupled plasma to ionize the samples.

Inductively coupled plasma optical emission spectrometry (ICP-OES) – an analytical technique that uses optimal emission spectrophotometer.

1.0 General introduction and thesis overview

1.1 Water in Alberta

Almost 80% of Alberta's surface water is found in the northern part of the province, flowing north towards the Arctic (Government of Alberta, 2010). One of the largest northward-flowing rivers is the Athabasca River, which flows through the Alberta bituminous sand region (ABSR). The Athabasca River is the longest and most studied river in Alberta (Donner et al., 2017; Kelly et al., 2010; Shotyk et al., 2017). Water quality of the Athabasca is vital as it subsequently feeds into the Peace Athabasca Delta, and Slave River, which then joins the Mackenzie River system, eventually draining into the Arctic Ocean (Government of Alberta, 2010). A 168 km section of the Athabasca River is designated as a Canadian Heritage River, a river reach that coincides with Jasper National Park, and empties into Lake Athabasca. The Athabasca River basin has a mean annual discharge of 45,900 x 10^6 m^3 (University of Alberta, 1990). The discharge from the Athabasca River forms a portion of the Peace Athabasca Delta which, is one of the largest freshwater deltas in the world, formed at its junction with the Peace River. This delta holds Ramsar site designation due to the size of the wetland. Approximately 390,000 hectares, is important heritage land of the Indigenous people, and a resting place for many species of migratory birds (Government of Alberta, 2010).

The Peace Athabasca Delta contains undisturbed grass and sedge meadows that provide valuable habitat for muskrat (*Ondatra zibethicus*), beaver (*Castor canadensis)*, waterfowl, and wood bison (*Bison bison athabascae*) (Government of Alberta, 2010). The Athabasca River contributes to a much larger ecosystem. The path of the Athabasca River coincides with the bitumen deposits in the ABSR and has the potential to become contaminated by vanadium and nickel because of the metals enrichment in bitumen (Dechaine and Gray, 2010; Lewan and Maynard, 1982; Schlesinger et al., 2017).

Tailings ponds are typically located along the Athabasca River and have elevated levels of nickel and vanadium from residual bitumen (Baker et al. 2012; Zubot et al. 2012). The Canadian Water Quality Guidelines for the Protection of Aquatic Life (CCME) do not have reported guidelines for vanadium quantities; however, nickel guidelines are estimated around 25µg/L (CCME, 2015). Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME) only allows for 45 mg/kg of nickel, and 130 mg/kg of vanadium in soil (CCME, 2015). It is important to determine non-intrusive methods of remediation, such as phytoremediation, to treat wastewaters and protect ecosystems like the Peace Athabasca Delta and the Athabasca River.

1.2 Phytoremediation

Phytoremediation is a form of remediation that employs planted vegetation to remove organic and inorganic pollutants from water and soils (Jeelani et al. 2017). Phytoremediation has been underutilized for remediating disturbed soils and water sources despite studies showing its effectiveness and low costs (Jeelani et al. 2017; Salt et al. 1995). In this study, I examined the ability of *A. calamus* to sequester vanadium and nickel. These metals were chosen for their enrichment in bitumen and their potential release into the environment through tailings (Shotyk et al., 2017). The results from this study will give insight into plant survival and potential impact on humans and local wildlife, including waterfowl and aquatic mammals.

Phytoremediation is a successful and economical option for the removal of heavy metals from wastewaters (Jeelani et al. 2017; Noor et al. 2017; Williams 2002). Several studies involving wetlands used specific plants to remove metals from wastewater. They have been shown to be successful in removing 50 to 99% of arsenic, cadmium, polycyclic hydrocarbons, and vanadium (Jeelani et al., 2017b; Noor et al., 2017; Sahu, 2013; Vachirapatama et al., 2011). In addition to regular phytoremediation, there are over 400 plant species classified as hyperaccumulators (Sun et al., 2013; Zhang et al., 2007). Hyperaccumulators are plants that extract extrodinarily high amounts of metals in the shoots of the plant, these concentrations being far in excess when comared to the majority of the species (Rascio and Navari-Izzo, 2011). Three basic distinguishers of hyperaccumulators are, enchanced rate of metal uptake, fast root to shoot allocation of metals, and an ability to store the metals in the shoots (Rascio and Navari-Izzo, 2011). Most hyperaccumulators exist in the *Alyssum* genus, yet, the *Acorus* genus has several hyperaccumulators as well. *Acorus calamus,* in particular, is a promising phytoremediation candidate and potential hyperaccumulator due to its robust root system, high adaptability, and extensive biomass (Sun et al. 2013; Zhang et al. 2007).

1.3 Acorus calamus

The species *A. calamus* occurs across a wide range, overlapping countries and human cultures, thereby earning several interchangeable names throughout its history: Sweet flag, Rat root, Vacha and many more depending on the country, language, and use (Motley, 1994). This plant is a herbaceous perennial with three varieties, diploid (*A. calamus* var. *americanus*), triploid (*A. calamus* var. *vulgaris*), and tetraploid (*A. calamus* var. *angustatus*) (Balakumbahan et al. 2010; Motley 1994). *A. calamus* is native to Alberta with the potential for modern wetland remediation because of its ability to tolerate polluted waters (Lansdown, 2014). This species grows in standing or slow-moving water sources, typically on the shorelines of marshes, mineral wetlands, lakes, ponds, and slow rivers (Lansdown, 2014). Jeelani et al. (2017) suggested that *A. calamus* could be useful for the extraction of pollutants from soils with multiple contaminants. The nature and rates of specific metal and nutrient uptake by the *A. calamus* are poorly known.

This plant has a wide range extending into 26 countries and has been introduced in 27 more (Figure 1), indicating its tolerance to a wide variety of growing conditions. *Acorus calamus* has not yet been grown commercially for reclamation processes (Lansdown, 2014). *A. calamus* is a robust species; labelled in North America as a zone 3 plant, indicating a high resistance to frost, cold temperatures, and fluctuating climates (Government of Alberta, 2017). Also, this plant grows in many types of soils and water, which makes it an excellent candidate for growth trials (Smreciu et al., 2015).

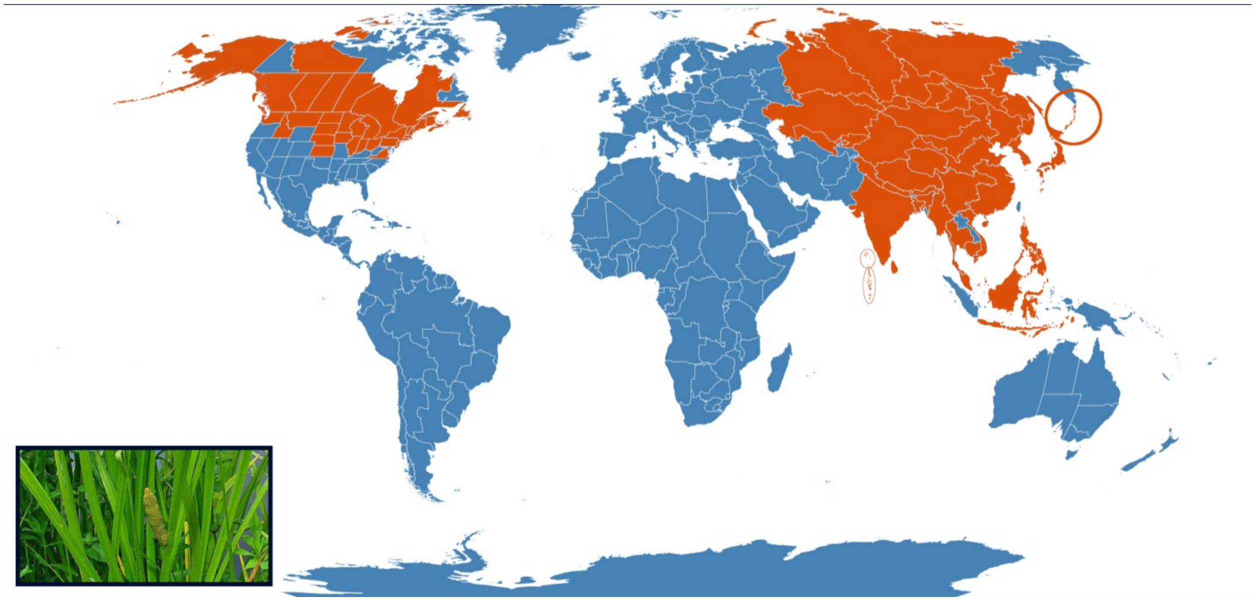

Figure 1. The native range of *A. calamus* is shown on the map in red. The map is provided by the University of Connecticut (University of Connecticut, 2020).

2.0 Thesis objectives

This study provides a detailed examination of the growth potential of *A. calamus* in cultivated conditions and its response when introduced to experimental water sources contaminated with nickel and vanadium. My two study objectives are: to examine the viability of the two primary methods of propagation and cultivation (seeds vs. rhizomes); and to clarify the capability of *A. calamus* to extract dissolved vanadium and nickel from experimentally treated waters.

Part One: Plant propagation

3.0 Acorus calamus propagation potential

3.1 Introduction

In this portion of the study, I examine *A. calamus* propagation methods to test viable options in a commercial setting to support wetland reclamation processes. I included both *A. calamus* seeds and rhizomes in wet VS. dry stratification trials. This species has two methods of spread, the first, sexual reproduction by seed from a seed head low on the stalk of the plant. The second (most commonly observed) through vegetative spread via rhizomes (Balakumbahan et al. 2010; Vojtíšková et al. 2004). Rhizomes are also the main medicinal attraction (Figure 24). Rhizomes have a white/pink interior which smells nutty and sweet when first opened, they also dry and store well, but can lose potency after drying (Smreciu et al. 2015).

3.2 History

Acorus calamus rhizomes were used in medicine as early as historical Greek and Roman cultures (Balakumbahan et al. 2010; Rajput et al. 2014). It has a wide range of uses throughout history, including the use of its citrus-scented leaves on the floors of institutions to ward off unfavourable smells and pests (Motley, 1994). The rhizome was later candied for European and early American delicacies (Motley, 1994).

Medicinally, *A. calamus* rhizomes were also used extensively by the Indigenous North Americans, Asian, and Indian cultures (Rajput et al. 2014). Rhizome oil, raw root, and dry powdered root were and still are the primary delivery methods for this type of medicine (Rajput et al. 2014). A study on oil extracted from *A. calamus* showed that its main ingredient, β-asarone, has a calming effect, which could account for the plant's many medicinal traits (Zanoli et al., 1998).

The earliest written documentation of *A. calamus* oil was in the Bible, which describes it as an essential oil in the making of Holy Oil (Exodus 30:23, 24, 34). Motley (1984) also mentioned that *A. calamus* grew in the famous gardens of Solomon and was a commonly traded item in the garden markets of Tyre. (Solomon 4:14; Ezekiel 27:19). The remains of *A. calamus* have been found buried in tombs throughout history, the most notable being that of King Tutankhamen (Manniche, 2009). In Indian markets around 371-287 BCE, *A. calamus* was reportedly used in the treatment of children's ailments such as stomach aches and colic (Barton and Castle, 1877).

Acorus calamus was transported from India to Russia and Poland during the conquests from 1237 to 1242, as it was believed to purify drinking water and was carried to new civilizations by the conquering people known as the Tatars (Motley, 1994). The plant became the foreboding symbol of conquest and became referred to as "Mongolian Poison" (Motley, 1994). During the Black Plague in Europe, *A. calamus* was reportedly used in a mixture called the "Vinegar of the four thieves": Distilled with other herbs and spices, thieves would drink and douse themselves in the concoction and remain unaffected as they proceeded to rob the houses of the diseased (Motley 1994). An early record of cultivation appeared in Vienna in 1574, and in 1588 it was recorded in Germany then introduced to France and Belgium by way of exchanges between botanical gardens

(Motley, 1994; Elliott, 1976). Throughout history, this plant was a valuable commodity, whether for its health benefits, its sweet flavour, its prolific yields, or its filtering qualities. Today it remains one of the most widely distributed medicinal plant species on Earth (Balakumbahan et al. 2010).

3.3 Islamic Gardens

Over 2500 years ago in ancient Persia, gardens were places to grow food, flowers, and medicinal plants; they were places for gathering, peace, and mindfulness as well as a place of spirituality (Jonathan, 2003). With the formation of Islam in the 7th century, Islamic-themed gardens were similar to those built in ancient Persia in that they were built as a place for spirituality, food, mindfulness, and as symbols of status (Jonathan, 2003). Islamic gardens were designed as a way to awe their visitors, provide food, and medicine (Harun et al., 2017). Gardens contained dynamic and geometric designs, succulent fruit, aromatic perfumes, and songbirds (Ruggles, 2008). *Acorus calamus* was introduced into Islamic gardens after the ages of conquests to purify the water, the focal element of the gardens (Motley, 1994). Soon after the introduction of *A. calamus*, it was made a staple plant in the gardens due to its many medicinal benefits and its sweet, nutty aroma (Rajput et al. 2014).

Acorus calamus holds significance to the culture and medicine of North American Indigenous Peoples, and the plant was used in practices predating the 12th century (Motley 1994; Zanoli et al. 1998). North American Indigenous Peoples use *A. calamus* in many forms, including raw, smoked, and for flavouring (Smreciu et al. 2015). Chewing or smoking, the raw roots are helpful when staving off hunger and fatigue (Motley, 1994). *Acorus calamus* has an extensive history in Alberta and around the world. Due to its widespread growth patterns and importance as a filtering plant, it is an excellent candidate for phytoremediation (Balakumbahan et al. 2010; Jeelani et al. 2017).

4.0 Methods

To examine the propagation growth patterns and growth requirements of *A. calamus,* I harvested approximately 20 plants at two sites along the south shore of Chip Lake, AB, on 10 September 2016, at 53º36'35"N 115º18'10"W and 760m of elevation. The plants bore mature seeds and were entering early seasonal senescence. The collection sites were 1 km apart. I cut the root masses out of the lakeshore using a shovel to disconnect the established masses, which were then stored in separate plastic buckets. I added 10L of lake water per bucket to protect excavated plants and to preserve the fine roots during transport. I brought the collected root balls to the University of Alberta Botanical Gardens and left them outside for a month in the fall until the leaves turned brown, showing that the plant had entered a state of full dormancy. After dormancy had set in, I washed the plants with reverse osmosis water, harvested the mature seeds and stored all rhizomes in typical wet, overwintering conditions.

4.1 Stratification

I removed the seeds from the bracts and grouped them into 100-seed sets before wrapping seeds in individual paper towel packets. I sealed each packet with paper clips on each end and either briefly soaked the packet in water or stored it dry, depending on the treatment. All seeds (300 damp, 400 dry) were stored in two separate black garbage bags, placed in a refrigerator to simulate overwintering conditions, and were left for six months.

Rhizomes were separated into two groups, those with emergent foliage and those with roots only. The rhizomes were washed thoroughly with reverse osmosis water, then separated and cut into smaller, manageable segments. Since rhizomes are the easiest way to propagate *A. calamus*, I applied two different treatments to the groups. Eighty rhizomes were viable, based on extensive foliage extending over 1 foot in length or ~80% of the rhizome section being covered in roots (roughly 20 or more individual roots). I divided the eighty segments randomly into two groups of forty rhizomes with each group containing twenty with foliage and twenty without foliage.

I placed the first group of seeds into seed trays, 54 cm x 28.5 cm x 5 cm, covered the trays in peat moss, thoroughly soaked them with reverse osmosis water, and set them outside for six months in a protected area (to prevent grazing). The rhizomes were covered with snow to insulate and mimic winter wetland conditions. The second group had each of the forty rhizome segments individually wrapped in wet paper towels, set into seedling trays, and set outside to freeze without any peat moss covering. The rhizome treatments were left to overwinter for six months.

4.2 Propagation

Propagation resumed on 30 March 2017, six months after the initial stratification. I transferred the seeds from their packets to seedling trays. I filled the trays with 5 cm of potting soil mixture, well-packed. I created five rows in each box then planted the seeds individually in each row using plastic tweezers and nitrile gloves. Each row contained 33 seeds set 1mm apart. I covered each row with

2.5 cm of topsoil. There were four trays with the damp seed treatment and two with the dry-stored seeds. There were six trays with 165 seeds per tray for a total of 990 seeds for this portion of the experiment. Each of the trays was punctured with holes in the base to allow water to flow through, mimicking the natural sub-irrigated ebb and flow cycle of a wetland. Planted trays were held at the greenhouse with temperatures fluctuating from 10 to 12 degrees Celsius, following outdoor conditions to best simulate a spring season for *A. calamus* seedlings (Motley 1994; Smreciu et al. 2015).

I moved the surviving plants into larger pots with more potting soil mix, put them outside, and thoroughly soaked them with reverse osmosis water. This process produced 156 young *A. calamus* plants.

After removing rhizomes from their respective boxes in the non-peat treatment, most rhizomes appeared to have not survived, appearing dried out and having no emergent foliage. However, 33 out of 40 survived and were successfully planted out.

5.0 Results

The seedlings began emerging on 28 April 2017, approximately one month after planting. On 18 May 2017, all damp-treated seeds (four trays) had successfully sprouted, and sprouts were roughly 2.5 cm tall while the dry seed treatments (two trays) showed no signs of life. Out of a total of 990 seeds, 156 damp treated seeds survived, and none of the dry treatment seeds sprouted. Seeds from the damp containers had a plant yield of 23.6% (Figure 2), indicating this seeding method of propagation was relatively inefficient for propagation on a large scale.

Out of a possible 80 rhizomes, 56 survived. Rhizomes remained in their separate treatments of peat moss VS. no-peat moss treatments until this point. Peat-covered rhizomes initially were thriving, yet, only 23 out of 40 plants survived. The rhizomes from the no-peat moss treatment still appeared dormant (they later recovered fully), while the peat moss treatment appeared to be highly successful as most rhizomes had new green growth. Similar to the seed treatment, rhizome treatments were each repotted into individual pots that I filled with potting soil mix, soaked, and placed outside. The rhizomes brought the total number of *A. calamus* plants to 212. The plants were left outside for the remainder of the summer to grow where they were watered every day. The results showed that rhizome trials without peat were the most successful, 82.5% yield compared to 57.5% in the with-peat treatment, as shown in Figure 2.

Plant yield, the ratio of plants that sprouted vs the entire population that could have sprouted, in % was calculated to determine the rate of seed and rhizome survival; the following formula was used.

$$\% \text{ yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100$$

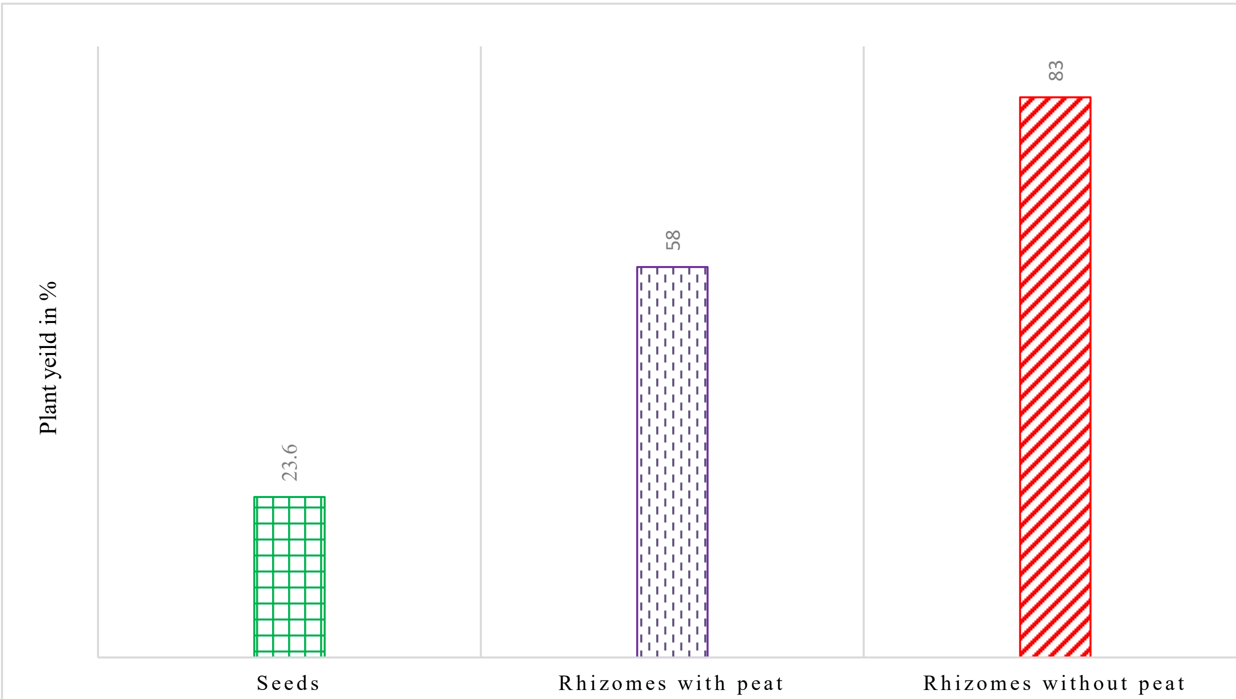

Figure 2. Plant yield (%) of *A.calamus* grown from seed vs. rhizome.

6.0 Discussion

Propagation of *A. calamus* using rhizomes is a more dependable growth technique compared to seed planting (Figure 2). This result was predicted due to the larger mass of nutrients and starches contained in the bulky rhizome that is needed to support new growth; this finding is consistent with earlier studies (Smreciu et al. 2015). Moist conditions provided the best storage method for growth in both seed and rhizome treatments. Natural *A. calamus* seed and rhizome propagation conditions are typically damp and dark environments, which was indicated in the experiments (Smreciu et al. 2015). Once mature and ripe, seeds from wild plants fall from the bract, land in the water where they float, slowly absorb moisture before sinking to the muddy bottom of the host wetland or water source, where they overwinter until spring (Smreciu et al. 2015).

The regeneration niche is a bottleneck in the life history of many wetland plant species (Grubb, 1977). It is also an important operational limitation should seeds fail to germinate or plant matter fail to survive during attempts at reclamation.

Rhizome cutting may mimic the relationship with root-grazers, such as muskrats (*Ondatra. zibethicus)* that excavate and feed wastefully. This process results in un-consumed root fragments being scattered and washing into new environments where they become new stands of *A. calamus.* In the laboratory, large roots can be cut into several viable rhizome portions, each containing viable buds and >5 roots on each segment, meaning multiplication of this species is relatively easy and straightforward.

The storage conditions before planting yielded unanticipated results. When the rhizomes were cold stratified outside without the added insulation of peat, they yielded higher success rates under subsequent propagation. A higher proportion (82%) of peat-less treatment survived compared to their insulated counterparts. The reasons for the higher mortality in peat-based storage is unclear, but there may be a life-history aspect if *A. calamus* happens to grow best in mineral soil conditions or if peat pH presents unseen complications. *A. calamus* is typically found in alluvial, loamy and loamy clay soils due to their porous nature and ability to retain water (Balakumbahan et al. 2010).

7.0 Conclusions

I compared two propagation methods for this species to assess its reclamation potential and found that the most predictable propagation technique for *A. calamus* was via rhizomatic vegetative spread.

Recommendations for large-scale vegetative propagation of *A. calamus* includes overwintering rhizomes buried in mineral soil and snow/ice, cutting rhizomes into 15-cm portions containing evident buds and healthy roots; then planting out in early spring after the rhizomes have broken dormancy.

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Part Two: Nickel and vanadium uptake from hydroponic solutions by <u>Acorus</u> <u>calamus</u>

8.0 Acorus calamus and its use in the environment

A. calamus acts as a natural filter that has been used historically in 53 countries to date (Motley, 1994). In several studies, *A. calamus* extracted polycyclic aromatic hydrocarbons, ammonia, and a suite of metals from wastewater sources (Jeelani et al., 2017; Noor et al., 2017; Sun et al., 2013). Although uptake of nickel and vanadium by *A. calamus* has not yet been evaluated, both are promising candidate metals for phytoextraction. Metals play an important role in physiology: nickel is an essential trace nutrient for animals and plants, while vanadium only appears essential for animals (French and Jones, 1993; Welch and Cary, 1975; Welch and Shuman, 1995). These metals play a significant role in the ecosystem and have been accumulating in humans and animals due to their increased use and occurrence in commonly used steel products (Schlesinger et al., 2017) and bitumen products (Huang et al., 2015).

Kelly et al. (2010) indicated that 13 elements (Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Ti, and Zn) listed as priority pollutants (according to the US EPA) were found in high concentrations close to the region of bituminous sands. Mining disturbance due to bituminous sands excavation was the main contributor of these pollutants to the Athabasca River (Kelly et al., 2010). In contrast, Shotyk et al. (2017) found only three abundant trace elements in the Athabasca River (V, Ni, and Mo).

8.1 Metals in the bituminous sands

Bituminous sand is a mixture of hydrocarbons, water, sand, and clay. Bitumen is enriched in four metals: molybdenum, nickel, rhenium and vanadium (Bicalho et al., 2017). Molybdenum and nickel are essential to plant and animal life in small quantities (French and Jones, 1993; Welch and Cary, 1975).

8.2 Vanadium

Vanadium has two stable isotopes, ^{50}V (0.25%), and ^{51}V (99.75%) (Huang et al., 2015; Rudnick and Gao, 2003; Schlesinger et al., 2017). This study uses ^{51}V for vanadium concentration measurements. In nature, vanadium is not found in its elemental state but occurs in 65 minerals (Lewan and Maynard, 1982). It is more abundant than nickel in the Upper Continental Crust in a 2:1 ratio of 135:75 ppm and is typically used to strengthen and reduce corrosion of steel and used as a specialty metal in batteries and electronics (Krupper and Kroneck, 2007; Schlesinger et al., 2017; Taylor, 1964). As referenced in the first chapter, tailings water has proven challenging to remediate though several methods have been tested over the years including, artificial membranes, precipitation, ion-exchange, residual coke, and centrifugal processes (Brewster and Passmore, 1994; Galil and Rebhun, 1990; Geckeler and Volchek, 1996; Zubot et al., 2012). With the ever-increasing demand for heavy, light, and unconventional crude oils, there has been a significant increase in vanadium mobilization (Schlesinger et al., 2017). Oil production in Alberta alone reached an annual production of 2.3 million barrels per day in 2014 (Government of Alberta, 2014).

8.3 Nickel

Nickel occurs in the Earth's Upper Continental Crust as a concentration of 75 ppm as elemental nickel and metal sulphides (Taylor, 1964). It is a silvery, white metal that has many applications in the modern world, including components of stainless steel, rust-resistant alloys and its use in batteries (Krupper and Kroneck, 2007; Rezania et al., 2016). Similarly to vanadium, nickel is abundant in bitumen, but it is also an essential nutrient for plants and animals (Vatansever et al. 2017; Welch and Shuman 1995). Nickel is becoming more abundant in the food system, agricultural soils, and tailings ponds due to anthropogenic activities. (Salt et al. 2000; Shahzad et al. 2018). Currently, there is more concern about nickel toxicity than nickel deficiencies (Krupper and Kroneck, 2007). Most anthropogenic nickel enters into the environment via heavy oil combustion, smelting, burning of coal, and wastewaters (Krupper and Kroneck, 2007). The examination of trapped material in peat and ice cores was used to date and determine trace metal pollution trends. It was determined that trace metal pollution started to increase during the Second Industrial Revolution (1850-1900); however, since the 1980s, atmospheric metal deposition has been decreasing.

9.0 Methods

9.1 Transplants

In the second study phase, I utilized the successfully propagated plants from the first portion of the study. I grew the plants in a metal-free, laminar flow clean-air cabinet, thereby ensuring minimal exposure to aerosols that could contain nickel and vanadium. I used the baseline values of the original plants to contrast against the experimentally treated plants. Some of the plants from the first study were grown outside of the clean-air cabinet and not treated with metal solutions to determine the effectiveness of the cabinet these are labelled as “wild”. In addition, control plants were grown inside of the clean-air cabinet and labelled “control” these plants were also not treated.

I washed the sections of *A. calamus* in a plastic bucket filled with reverse osmosis water. Once rinsed, I used a ceramic knife to separate the rhizomes into several viable parts, depending on the size of the original plant. I trimmed roots and shoots back, to give way for new growth and to fit into the new, rinsed, 5 x 5 cm containers. The plants were grown in acid-washed quartz sand (washed with 1% nitric acid) to provide an inert and contamination-free medium. After I moved the new rhizome segments into the pots, I covered them with approximately 5-cm of the inert quartz sand that acted as the growth medium. The rhizome segments were transferred into larger (15.7 x 12.7 cm) polyethylene containers to create a composite and redundant sample of three plants (within one sample unit) to ensure redundancy in the event of mortality of some roots (Figure 9). I moved all sample units into the clean-air cabinet and filled each unit to a depth of 3.5 cm of water from the reverse osmosis system at the University of Alberta Botanical Gardens. Full-spectrum grow lights were hung on three sides of the clean-air cabinet before placing the plants inside and adding aluminum foil to the fourth side to ensure equal distribution of heat and reflected light.

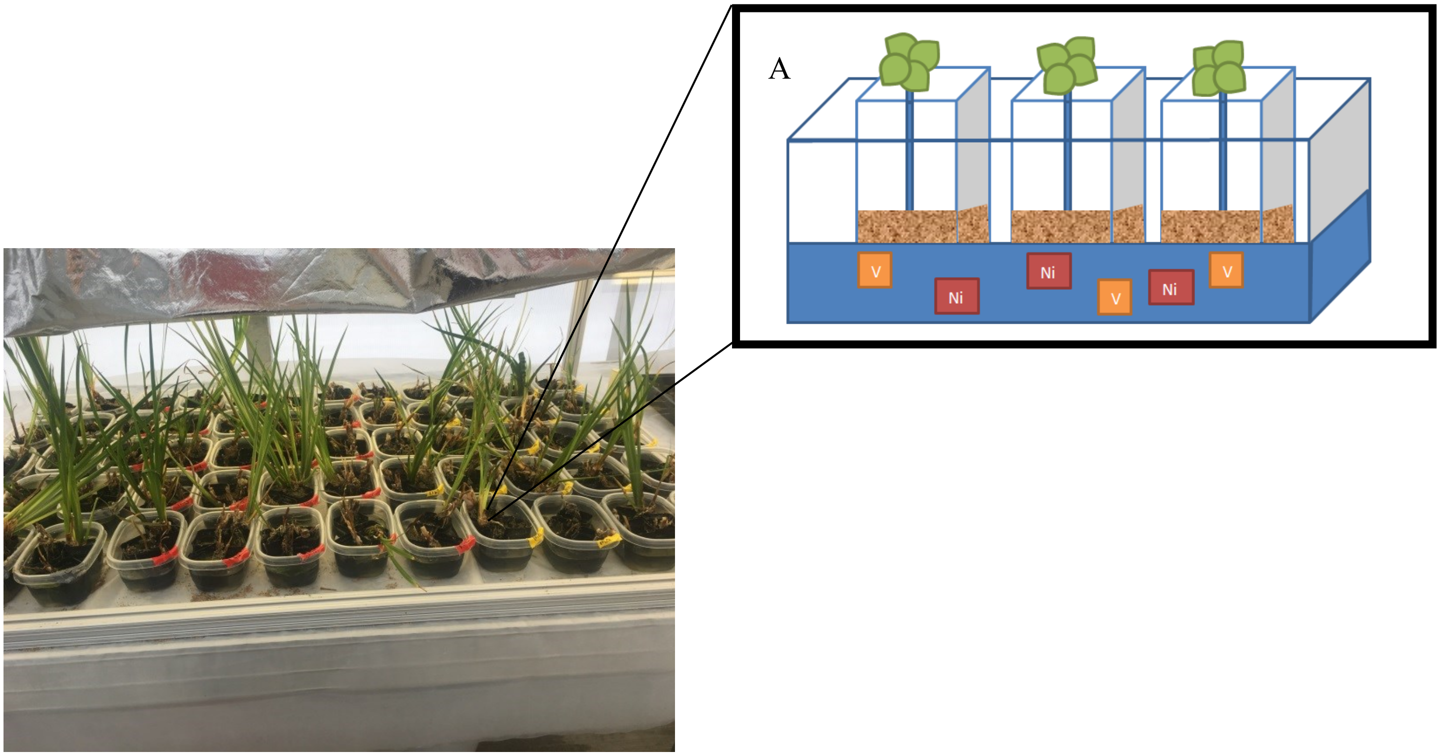

Figure 3. Sample design included one sample unit (picture A) containing a three-plant design to accommodate plant mortality.

10.0 Plant material and growth conditions

10.1 Growth chamber

The remainder of the study involved growing and treating the plants inside the clean-air metal free laminar flow cabinet. The cabinet as built at the University of Alberta and brought out to the University of Alberta Botanical Gardens for this portion of the study. The clean-air cabinet had acrylic walls that allowed optimal light saturation from the surrounding growth lights (Figure 10). These walls were set in an aluminum frame to house the plants while they grew. The top was retrofitted polypropylene plastic that housed a High-Efficiency Particulate Air (HEPA) filter rated to remove 99.97% of air particles > 0.2 microns in size. The clean-air cabinet dimensions were 96.5 cm tall and 152.4 cm wide which significantly limited the workspace and thus, was the limiting factor for the 5 x 5 cm pots in this study. Cabinet dimensions only allowed for a total of 60 sample units, arranged in 12 rows of five units. Three full spectrum grow lights were placed around the exterior of the cabinet to help the growing process. The cabinet was allowed to fluctuate temperature with the greenhouse however during the day it ranged from 23-27°C and dropping to 19-23°C during the night.

Figure 4. Clean air growth chamber, with HEPA filters on top and laminar air flow through front panel.

10.2 Nutrient solutions

Hoagland's basalt #2 solution (Welch and Cary, 1975) was the nutrient addition I used to fertilize the plants. This solution was used in similar studies due to it containing the essential nutrients for plant survival (Silva and Uchida, 2000; Singh and Wort, 1969; Vojtíšková et al., 2004). The Hoagland's solution was obtained from Caisson Labs Inc. in February 2018. The nutrient mixture was tested in the Natural Resources Analytical laboratory (NRAL) at the University of Alberta using a inductively coupled plasma optical emission spectrophotometer (ICP-OES) to determine a suite of ten elements (Na, K, Ca, Mg, Zn, Fe, Mn, Cu, S, P) (Figure 11).

10.3 Nickel and vanadium Treatments

In this portion of the study, I examined plant shoots exposed to nickel and vanadium, at three concentrations to measure the quantity taken up by the plant and stored in the shoots. Vanadium treatment concentrations were; low (no added vanadium), mid (0.025mg/L), and high (0.25mg/L). The mid and high concentrations were significantly higher than values (0.23 µg/L) found in the Athabasca River (Shotyk et al., 2017). However, vanadium and nickel have been found in high concentrations in dry sources, a typical petroleum coke (a byproduct of bitumen upgrading), for example, contains 1,680 ppm vanadium and 500 ppm nickel (Gosselin et al., 2010). They have been found in very low concentrations in water sources due to the chemical interaction with the carbon matrix. This interaction renders the elements inaccessible by water, averaging only 0.26 ppm vanadium and 0.04 ppm nickel in leachate (Baker et al., 2011; Gosselin et al., 2010); thus, by using these values compared to values in the wetland, this experiment resulted in up to 10x the amount found in similar studies using dry materials (Baker et al., 2012; Simhayov, 2017).

I pre-mixed solutions of nickel oxide and vanadium oxide in 2% nitric acid for this experiment to improve solubility of the metals in solution. All dilutions were prepared in the Soil, Water, Air, Manure, and Plants (SWAMP) clean lab at the University of Alberta. Dilutions were made using Eppendorf pipettes and pipetted into sterile volumetric containers, then sealed with lids, and parafilm. Vanadium solutions had to be wrapped in light impenetrable cloth due to the solutions' sensitivity to light and significant degradation with exposure. Nickel had no special requirements for transportation. All tubes were transported in upright tube racks, held in sealed plastic bags, placed into a cloth-lined box, and transported to the University of Alberta Botanical Gardens.

At the greenhouse, I rinsed five, 20 L containers to use as vessels for mixing the nickel and vanadium solutions. The medium value of [0.01mg/L] nickel was mixed into one of the 20L containers, and containers were rinsed three times with reverse osmosis water to ensure the entire solution was added. I filled the container halfway with reverse osmosis water before mixing in the Hoagland's solution to fully incorporate the mixture into the nickel/water mix and to ensure a homogenous solution. I repeated these steps for the five treatments; the control also followed the same method without any metals being added. I labelled each container appropriately, and I created an opaque container with a black garbage bag to protect the integrity of the vanadium solution.

10.4 Treatment strategy

I removed all the pots from their sample units and rinsed each with reverse osmosis water so they would have less risk of contamination. I rinsed all sample containers thoroughly, let them dry, and created tags for the plant pots with individual numbers that I randomized with a random number generator. The same randomization occurred for the sample units and treatments to restrict sample bias. Lastly, I colour-coded and marked all containers by metal treatment.

I placed all labelled samples in the clean air cabinet. Each plant was planted in the inert quartz sand growth medium. Every day additional water was added to each treatment unit with designated watering cans, and each unit was moved one spot forward to allow each plant to adjust for any variation of illumination or air movement within the clean-air cabinet. Each week I removed all plants, rinsed them individually in reverse osmosis water, cleaned the containers, and placed the sample units back with new solutions to prevent any accumulation of metals due to evaporation (Welch and Cary, 1975).

10.5 Sampling procedure

I sampled plants after 30 days of the treatments. I removed all samples from the respective containers using a ceramic knife and nitrile gloves to prevent contamination. After removing all shoot material, I individually rinsed the pieces with reverse osmosis water and placed them into labelled sterile bags. I bagged all the material and put it into a freezer to preserve the plant tissue before weighing and drying. I stored the samples in the freezer for two days and individually weighed each sample at the SWAMP preparation lab. I placed all samples in an oven at 105ºC for 48 hours; once all samples were thoroughly dried and cooled, they were removed and weighed once more to determine water content. I used a centrifugal ball mill with agate jars to mill all the samples; the mill ground four samples at a time working in alternating directions for 10 minutes at 2 min intervals to achieve uniform homogeneous mixtures. The mill was cleaned thoroughly in between each sample to prevent cross-contamination. After milling, I sent all samples to the University of Alberta SWAMP lab for acid digestion using 3ml HNO_3 and 0.1ml HBF_4 to create a final volume of 10 ml per sample.

10.6 Chemical analysis of plant material

Samples were analyzed on an ICP-MS (at the SWAMP laboratory), which provided the necessary analytical sensitivity for vanadium and nickel in plant materials (Krupper and Kroneck, 2007; Vachirapatama et al., 2011). The samples were run at two levels of dilution to extract the most representative values possible. Samples were run twice, at 10-fold dilution and 100-fold dilution to minimize the matrix effects and to double-check values near the limit of detection (LOD). The LOD, was determined using five blanks of 2% HNO_2.

The standard reference material for this experiment was NIST SRM 1547 (Peach Leaves). Standards were run four times at three different dilutions (10, 100, 1000x), spaced evenly throughout testing to ensure that the recovery was accurate over the range of sample concentrations. All samples analyzed were in quality control blocks, which reduces the risk of inaccuracy due to the concentration range of these samples.

11.0 Results

Results from the well waters at the University of Alberta Botanical Gardens showed elevated levels of calcium, magnesium, sodium, potassium, sulphur, and iron (Figure 11). However, after being run through a reverse osmosis treatment system, the hose water in the greenhouse only showed elevated levels of sodium. I determined this water suitable for the study parameters.

I tested two nutrient solutions to determine the appropriate concentrations for this experiment; I chose both due to their reported ability to function well in hydroponic conditions (Lee et al., 2008). The nutrient solutions were analyzed using an ICP-OES, which showed that Hoagland's solution contained elevated levels (Cu, Ca, Fe, K, Mg, Mn, Zn, Na, S, P) compared to Pro Grow (Figure 11). After choosing Hoagland's solution, it was analyzed further using an ICP-MS to determine the concentrations of vanadium and nickel contamination in the nutrient solution (Figure 12).

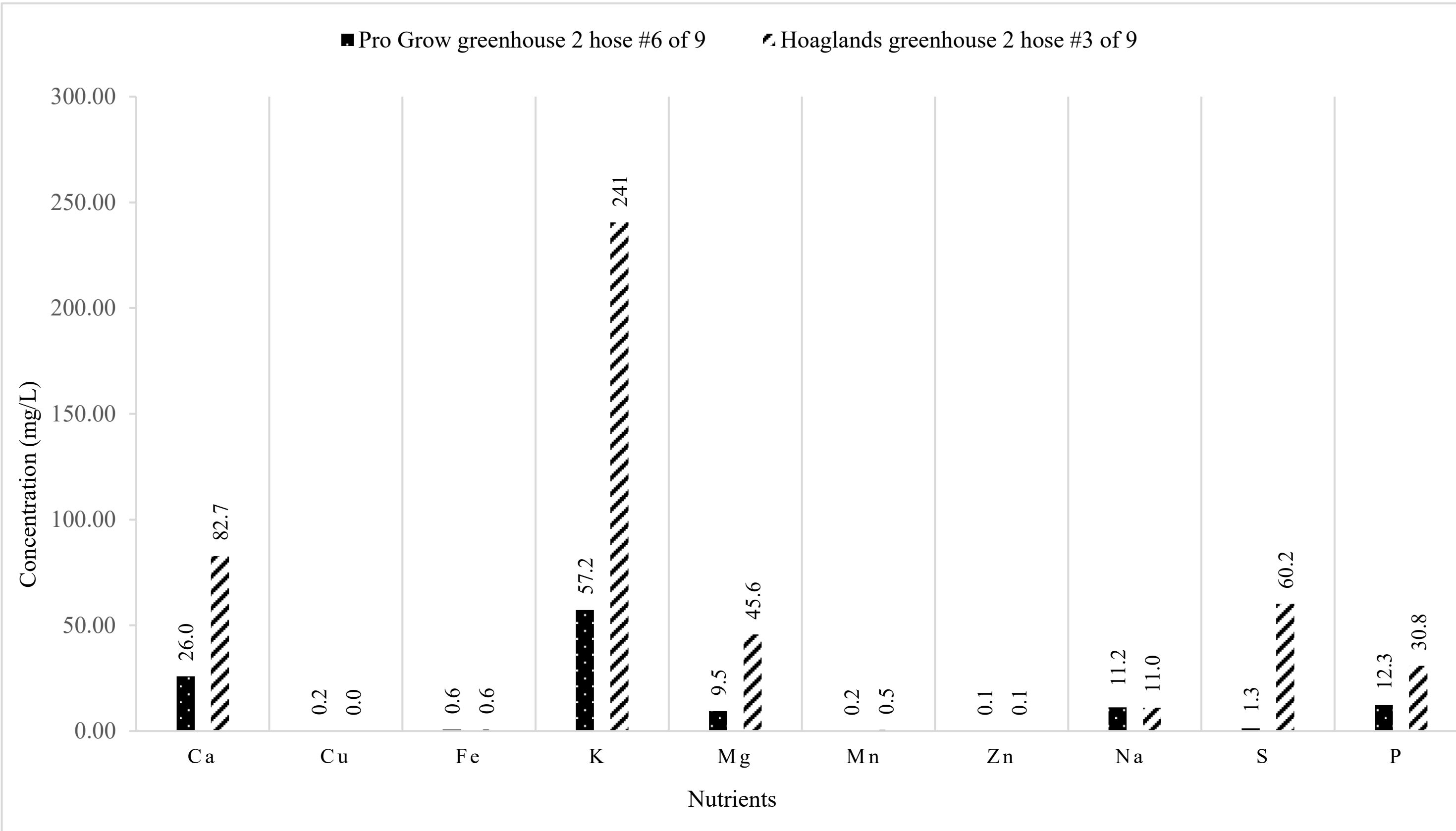

Figure 5. Major element concentrations (mg/L) in Pro Grow and Hoagland's solutions. Samples were measured using ICP-OES.

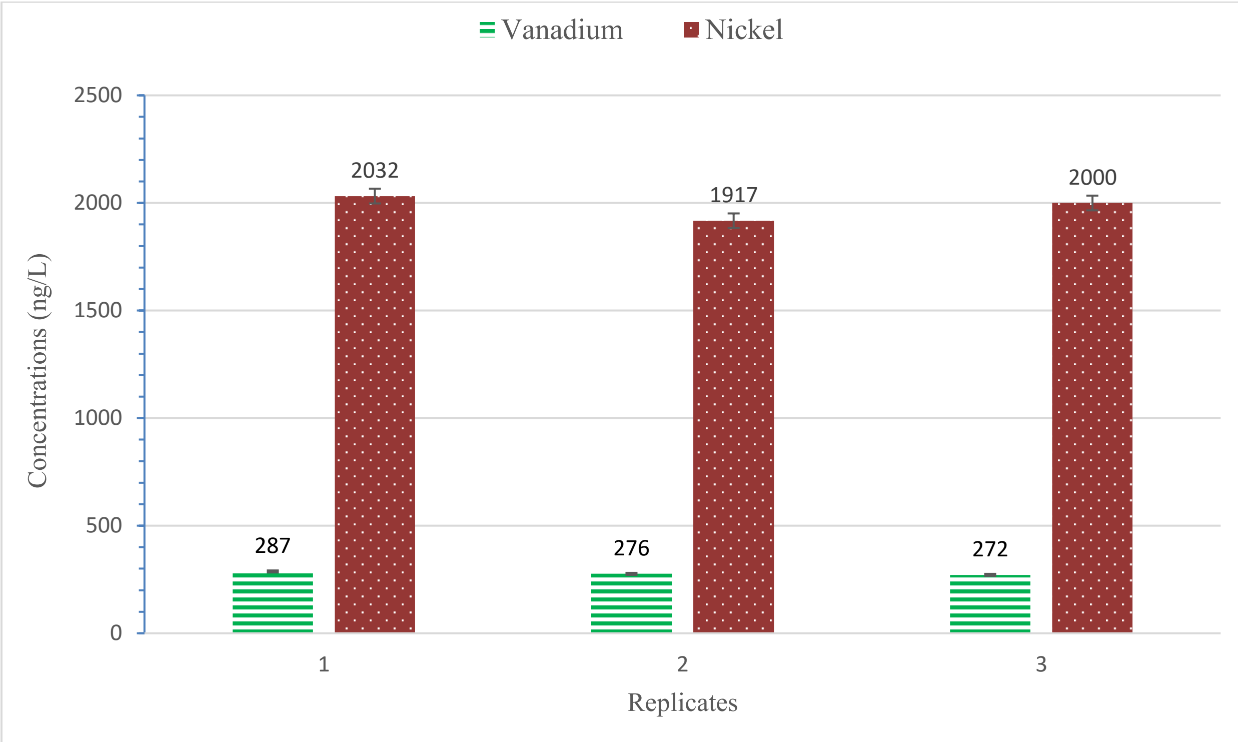

Figure 6. Concentrations of vanadium and nickel in Hoagland's solution in ng/L. Samples were measured using ICP-MS. The mean vanadium concentration and standard deviation in solution was 278 ng/L and 6 respectively, and the mean nickel concentration and standard deviation in solution was 1980 ng/L and 48 respectively.

11.1 Data analysis

I analyzed the results and created ANOVA's with the data sets to determine their significance. (R studio Version 1.0153) using lsmeans, cowplot, multicompview, plyr, tidyr, dplyr, and ggplot2. All data was analyzed using normal distributions. All data was compared against vanadium and nickel base values found occurring as trace elements in the Hoagland's solution. The results of the ANOVA showed the nickel mid treatment level to be statistically significant, whereas the high treatment level was not (Table 2). The ANOVA performed on the vanadium data showed the high treatment level is statistically significant and the other treatment levels were not (Table 3).

Treatment	**Group**
Ni Wild (not inside cabinet, 0 mg/L)	d
Ni Control (0 mg/L)	a
Ni Mid (0.01mg/L)	**b**
Ni High (0.1mg/L	a

Table 2. Statistical significance of nickel in plant shoots, , using a one way ANOVA. Letters that are the same are of no statistical significance, letters that are different are statistically significant.

Treatment	**Group**
V Wild (not inside cabinet, 0 mg/L)	b
V Control (0 ml/L)	ab
V Mid (0.025 mg/L)	a
V High (0.25mg/L)	**c**

Table 3. Statistical significance of vanadium in plant shoots, using a one way ANOVA. Letters that are the same are of no statistical significance, letters that are different are statistically significant.

11.2 Nickel

Phytoaccumulation of nickel showed similar results between the high and mid-level treatments; the nickel concentration in the plant shoots is shown in Figure 13. At the highest treatment level (0.1mg/L), *A. calamus* accumulated a mean of 12.2 mg/kg in the shoots, with the highest concentration being from the mid-level treatment (0.01mg/L) at 16.3 mg/kg (Table 1). The mid-level treatment showed an mean concentration in shoots of 6.8 mg/kg (Table 1). Nickel shoot concentrations in both high and mid-level treatments grouped around the 0-10 mg/kg range. However, both high (0.10mg/L) and mid (0.01mg/L) concentrations in shoots exceed the control and wild samples (Figure 13). Nickel in this study was in much higher treatment levels (10-fold increase) compared to natural amounts found in the Athabasca River, tailings ponds, and wetland studies (Figure 15). Nickel distribution coefficients for each level of treatment showed the lower the level of concentration, the more efficient the plant became at extracting the nickel (Figure 17).

11.3 Vanadium

Vanadium showed significant differences between controls and the high treatments, however not at mid-level treatments. The vanadium concentration data is shown in Figure 14. Vanadium was removed by *A. calamus* up to 0.60 mg/kg in the shoots at the highest treatment level and had a mean of 0.32 mg/kg found in the plant shoots (Figure 14). At the mid-treatment level, *A. calamus* accumulated a mean of 0.07 mg/kg. Vanadium high treatment is visibly grouped around 0.4-0.8 mg/kg, surpassing mid (0.025mg/L) vanadium, wild (0.mg/L), and control (0mg/L) treatments shown in Figure 14. Vanadium concentration at all treatment levels, when compared to tailings ponds, the Athabasca River, and wetlands, was found to be of elevated levels in this study (Figure 16).

Concentration (mg/kg)

Samples	Highest	Lowest	**Mean**	**SD**
Ni Wild	0.5	0.1	**0.3**	**0.1**
Ni Control	16.4	0.4	**4.7**	**0.62**
Ni Mid*	16.3	0.9	**6.8**	**5.80**
Ni High	12.2	1.1	**5.7**	**3.37**
V Wild	0.0	0.0	**0.0**	**0.005**
V Control	0.5	0.0	**0.1**	**0.15**
V Mid	0.4	0.0	**0.3**	**0.11**
V High*	0.6	0.1	**0.1**	**0.14**

Table 1. Concentrations of nickel and vanadium in plant shoots. Values represent the mean of 12 samples analyzed using ICP-MS. Statistically significant treatments ($P<0.05$) from a one way ANOVA are shown with *.

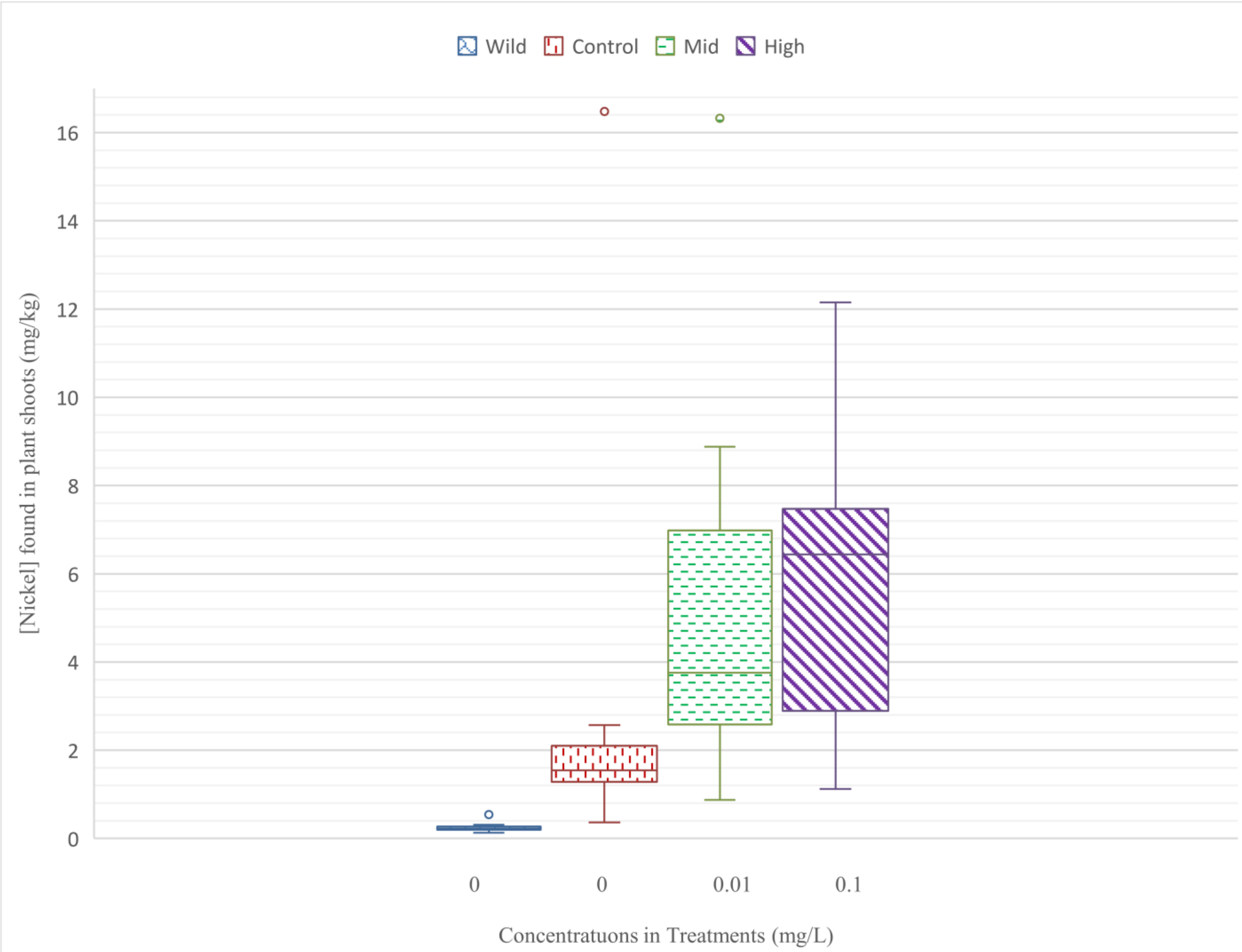

Figure 7. Display of nickel accumulation in *A. calamus* shoots. Pattern codes are: blue = wild plants, red = control treatments, green = nickel mid treatments, purple = nickel high treatments. Statistically significant treatments ($P<0.05$) are shown with *.

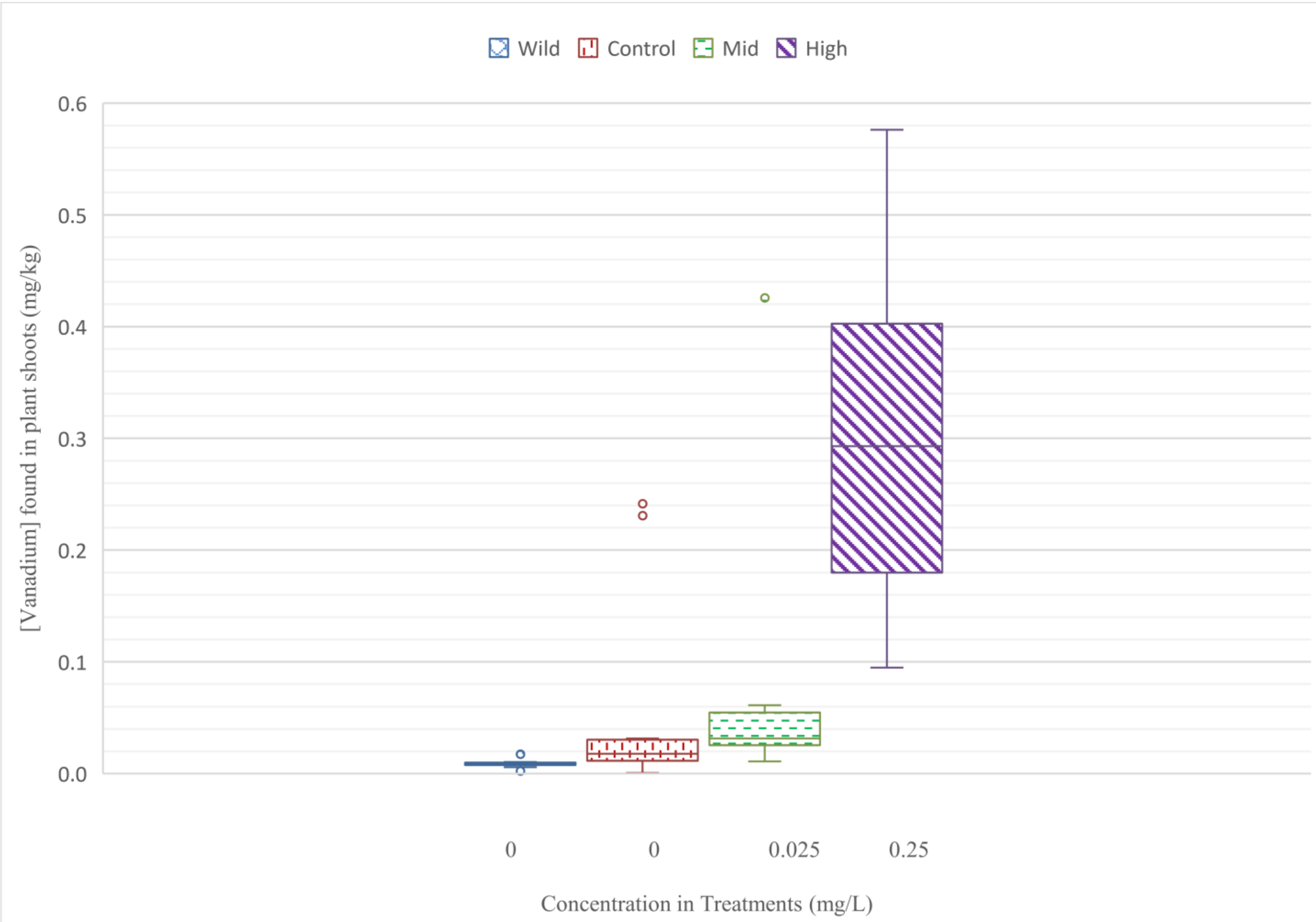

Figure 8. Display of vanadium accumulation in *A. calamus* shoots. Pattern codes are: blue = wild treatments, red = control treatments, green = vanadium mid treatments, purple = vanadium high treatments. Statistically significant treatments ($P<0.05$) are shown with *.

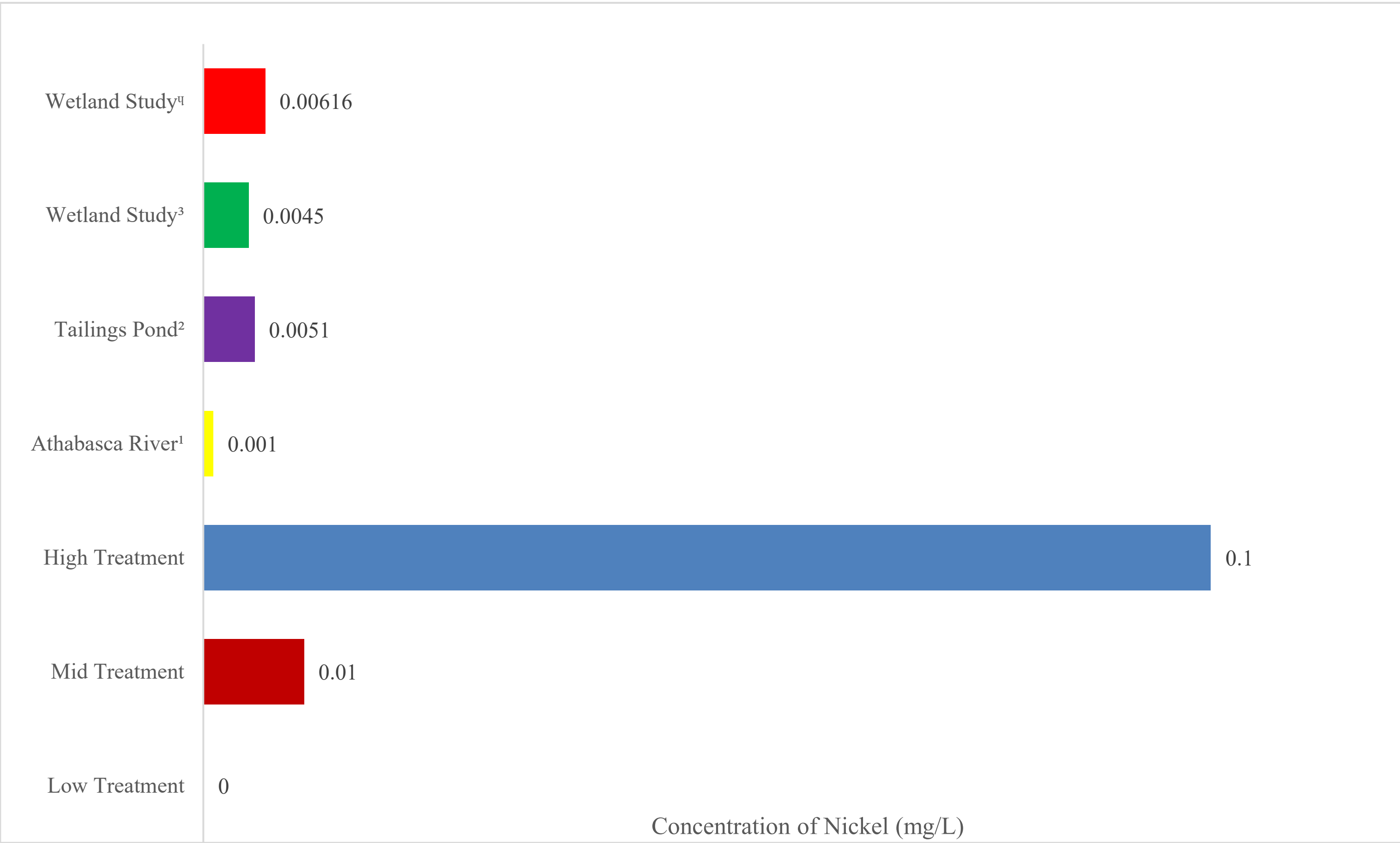

Figure 9. Concentrations of nickel in the treatments compared to water sources in Alberta. (Shotyk et al., 2017)[1], (Baker 2012)[2,3], (Pourret et al., 2011)[4].

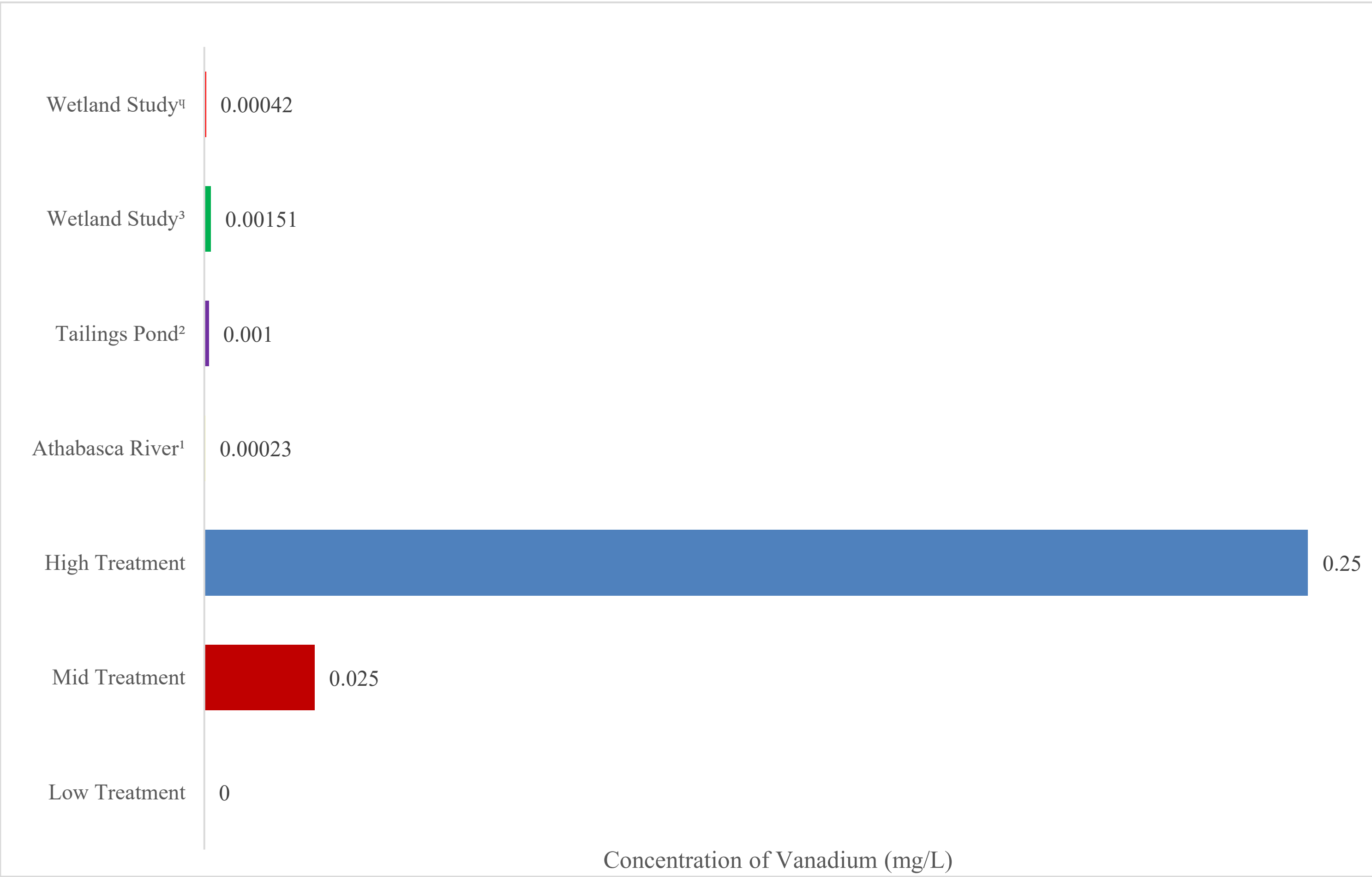

Figure 10. Concentrations of vanadium in the treatments compared to water sources in Alberta. (Shotyk et al., 2017)[1], (Zubot et al., 2012)[2], (Pourret et al., 2011)[3], (Schiffer and Liber, 2017)[4].

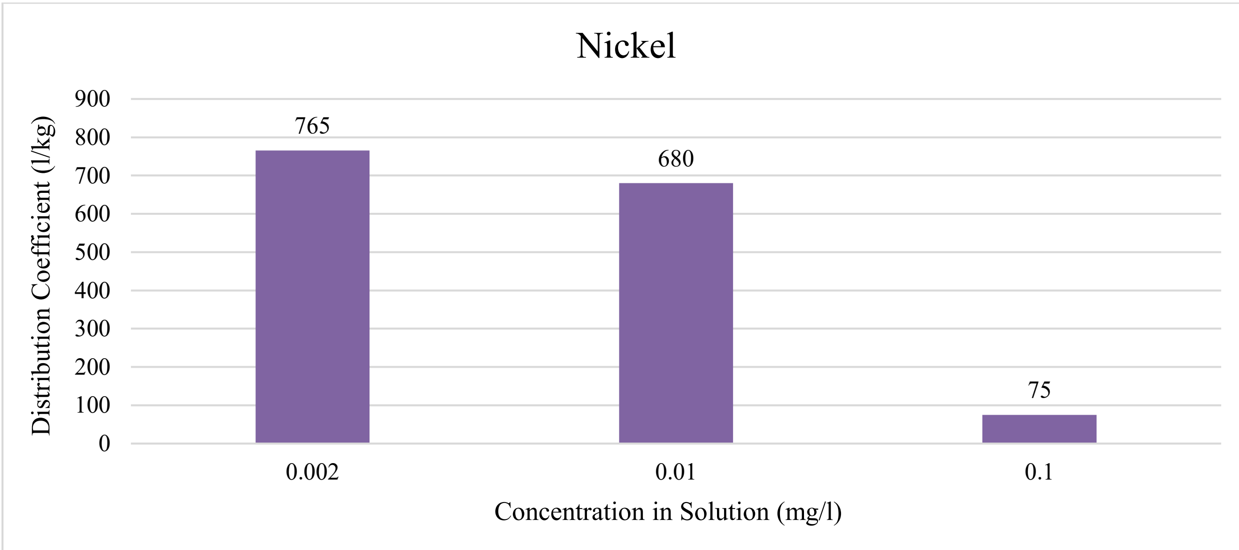

Figure 11. Distribution coefficient ratio of solid phase to aqueous phase concentrations of nickel at low (0mg/L), mid (0.01mg/L), and high (0.10mg/L) treatment levels.

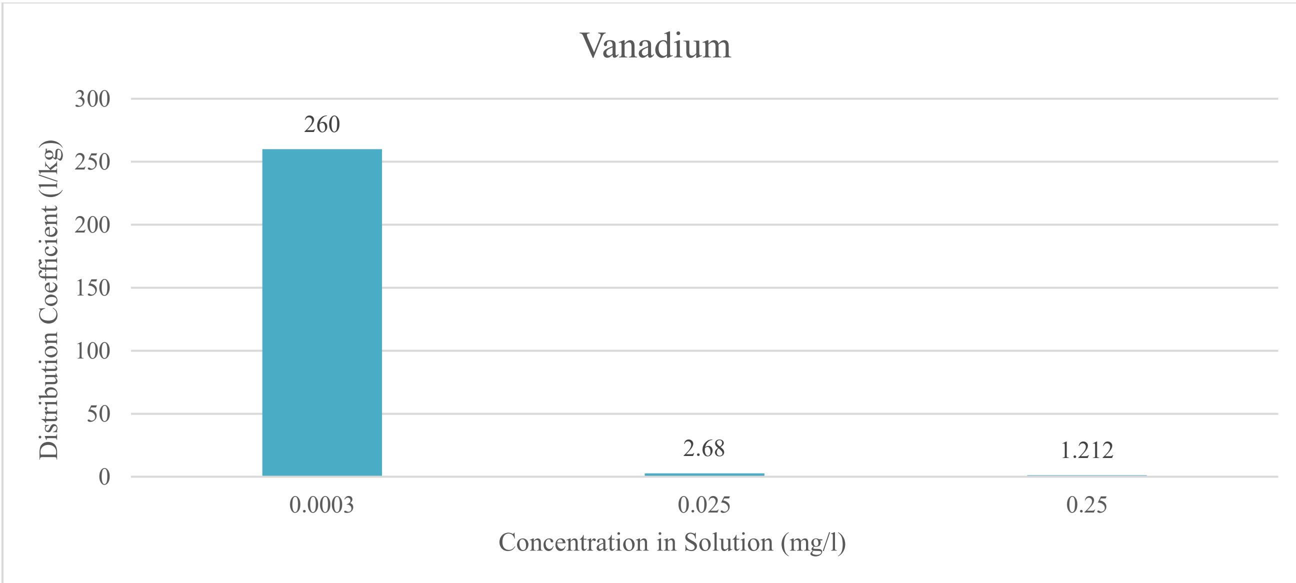

Figure 18. Distribution coefficient ratio of solid phase to aqueous phase concentrations of vanadium at low (0mg/L), mid (0.025mg/l), and high (0.25mg/L) treatment levels.

12.0 Discussion

12.1 Plant growth

After 30 days of growth, the plants showed yellowing of leaves in all treatments, which could have been due to sunlight overexposure, nutrient overloading, or natural senescence patterns. The plants also had a natural cycle in which leaves would grow, become yellow, and then the plant would produce new shoots to replace the old; this occurred regularly before any treatment was added to the samples. There was observable algal growth in all treatments with no apparent preference for any specific treatment. Though not directly measured, I did not observe a detectable difference in root to shoot biomass between any of the treatments. Another study showed contaminants (cadmium, and polycyclic aromatic hydrocarbons) affected the plant biomass, suggesting that when contaminants are in combination with nutrients, they can provide many different responses (Jeelani et al., 2017).

12.2 Metal uptake

Acorus calamus has shown it is a promising candidate for remediating vanadium and nickel. It is effective in the extraction of contaminant levels below and above 0.25mg/L and 0.10mg/L from water sources (Figures 17 and 18). Due to this *A. calamus* would be useful as a precursor management technique before chemical treatment or as initial treatment of surface water in tailing ponds. Levels of nickel and vanadium can range in tailings ponds from 0.0051mg/L and 0.001 mg/L in the dissolved fraction (Baker et al., 2012; Rezania et al., 2016; Zubot et al., 2012).

I found that *A. calamus* extracted up both vanadium and nickel in all treatments. The wild plants had small concentrations of vanadium and nickel levels likely due to the growing conditions outside of the clean air cabinet (Figures 13 and 14). The control plants showed elevated levels of both vanadium and nickel when compared to wild plants; unlike the wild plants, this contamination is unlikely to be due to aerial contamination. The only obvious source of nickel and vanadium in the controls is due to small concentrations of vanadium and nickel present in Hoagland's solution (Figure 12).

The results of this study were compared to naturally occurring nickel and vanadium concentrations in the Athabasca River downstream of the ABSR excavation sites (Shotyk et al., 2017) (Figures 14 and 15). Treatment levels used in this experiment were well above the naturally occurring metal concentrations found in Alberta's waters (Figures 14 and 15)(Baker et al., 2012; Pourret et al., 2011; Schiffer and Liber, 2017; Shotyk et al., 2017; Zubot et al., 2012).

After determining that *A. calamus*, when exposed to the vanadium mid treatments, had the highest shoot concentrations, efficiency of *A. calamus* was examined and showed that the plant was extremely inefficient at accumulating vanadium (Figure 18). Similarly, with nickel concentrations, as the concentration of the metal treatment increased, the efficiency of *A. calamus* accumulation decreased (Figure 17).

Vanadium concentration in the shoots, when compared to the associated wild treatment, showed both mid (0.025mg/) and high (0.25mg/L) treatments having elevated metal values. However, vanadium high (0.25mg/L) was the only treatment significantly different from the low (0mg/L) and mid (0.025mg/L) treatments, despite being inefficient at accumulation (Figure 18). The mass of uptake may be due to vanadium in solution in the form of vanadate, which is chemically similar to phosphate and necessary for plant nutrition (Crans et al., 2004). It is likely the plants were mistaking the similar ions and were taking up vanadium in lieu of phosphate (LeSueur and Puckett, 1980). If the plants were phosphate-limited, the uptake of vanadium would accumulate in greater amounts. (Crans et al., 2004).

Acorus calamus extracted nickel during the treatments; however, the plant appeared to have extracted the nickel as a means of elemental necessity rather than acting as an accumulator, therefore accumulating it in small quantities rather than rapidly accumulating it in an abnormal amount. The nickel mid and high treatments did not differ significantly from each other. However, in both mid and high-level treatments, *A. calamus* appeared to have extracted nickel from the water source up to 10 mg/kg. Nickel uptake by *A. calamus* in high treatment levels showed a mean of 6.3 mg/kg, representing a small increase over the mid-level treatment plants. Similar studies have found varying results showing that the nickel quantity in the plant is directly correlated to the quantity in the nutrient solution (Figure 13) (Welch and Cary, 1975).

This study shows that *A. calamus* can effectively remove a large range of vanadium (±0.25mg/L) and nickel (±0.10mg/L) concentrations from contaminated water.

13.0 Conclusion

Acorus calamus has been found to take up both nickel and vanadium from solution. While nickel is essential to plants, vanadium is not. Given the chemical similarity of vanadium and phosphate, uptake of vanadium in this study is likely passive sequestration by the plants. The abilities of *A. calamus* to operate effectively to remove these metals from water at low concentrations could indicate that this plant can extract higher concentrations of vanadium and nickel than were ever found in the water in which they grow (Shotyk et al., 2017). *A. calamus* would be effective in the removal of vanadium and nickel if all other conditions are suited to the plant's natural living conditions.

14.0 Synthesis

Phytoremediation describes the technique of using planted vegetation to remove organic and inorganic pollutants from water and soils. Phytoremediation has been underutilized for remediating disturbed soil and water sources despite studies showing its effectiveness (Jeelani et al., 2017; Salt et al., 1995). *Acorus calamus* has promising extraction abilities.

Due to the active exchange and naturalization of this plant globally and its extensive historical value as a filtering plant, it is an excellent candidate for remediation and growth trials. It is an easily transplanted and propagated plant. Chapter 1 results showed rhizomes stored in cool, damp conditions achieved successful propagation rates the following spring. This method of propagation could be utilized on a much larger scale for remediation purposes.

In Chapter 2 results showed the uptake of vanadium and nickel from water sources by *A. calamus* was highly successful depending on exposure concentrations. *A. calamus* accumulation of nickel and vanadium in the shoots exceeded the metal concentrations found in Alberta's waters (Figures 15 and 16) (Baker et al., 2012; Pourret et al., 2011; Schiffer and Liber, 2017; Shotyk et al., 2017; Zubot et al., 2012). Accumulation was more pronounced in the high (0.25mg/L) treatment levels of vanadium and the mid (0.010mg/L) levels of nickel. *A. calamus* would be successful in extracting these metals in contaminated areas that exceed the experimental treatment levels in this study. Shoot concentrations of both metals were elevated in comparison to natural values (Figures 15 and 16).

Recommendations for next steps would involve field trials with *A. calamus* in known contamination sites with quantified concentrations of metals to get a better understanding of the plant's relationship with vanadium and nickel in the environment. Constructed wetlands with controlled water sources could be planted with *A. calamus,* have added contamination sources (vanadium and nickel), and be measured over time. This experiment might help corroborate the patterns observed in the laboratory and greenhouse. The fate of metals taken up remains uncertain. If the above-ground foliage is clipped and removed, some metals will be taken with the clippings. The bioavailability of vanadium and nickel in plant tissues is unclear. If the metal-enriched leaves are allowed to senesce and decompose on-site, the metals may become available in the water column again, or they may become deeply buried within the organic matter.

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Appendix

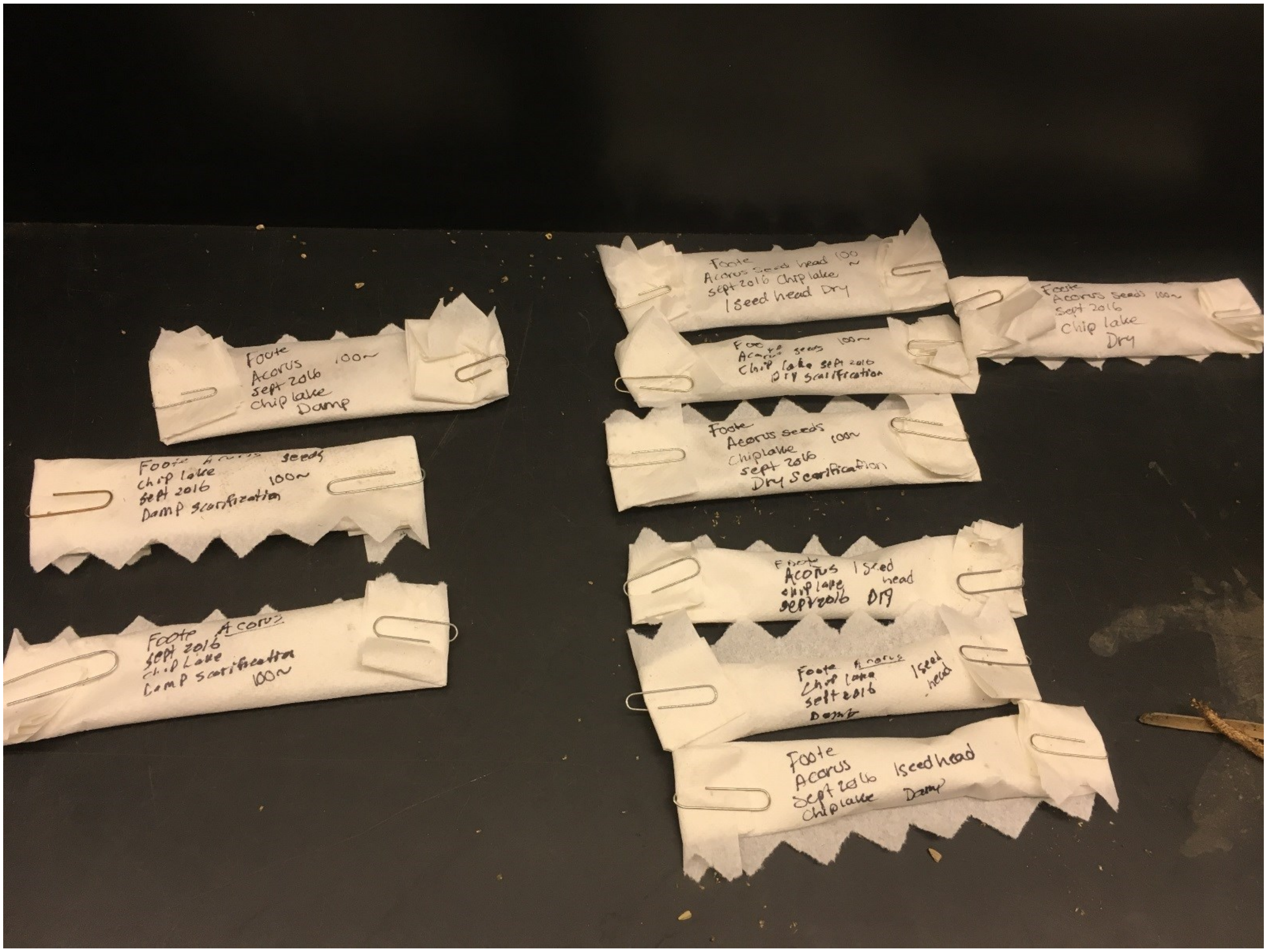

Figure 12. Initial seed packets ~100 per package stored either damp or dry.

Figure 13. Damp seed packets after storage in the refrigerator in a black plastic bag.

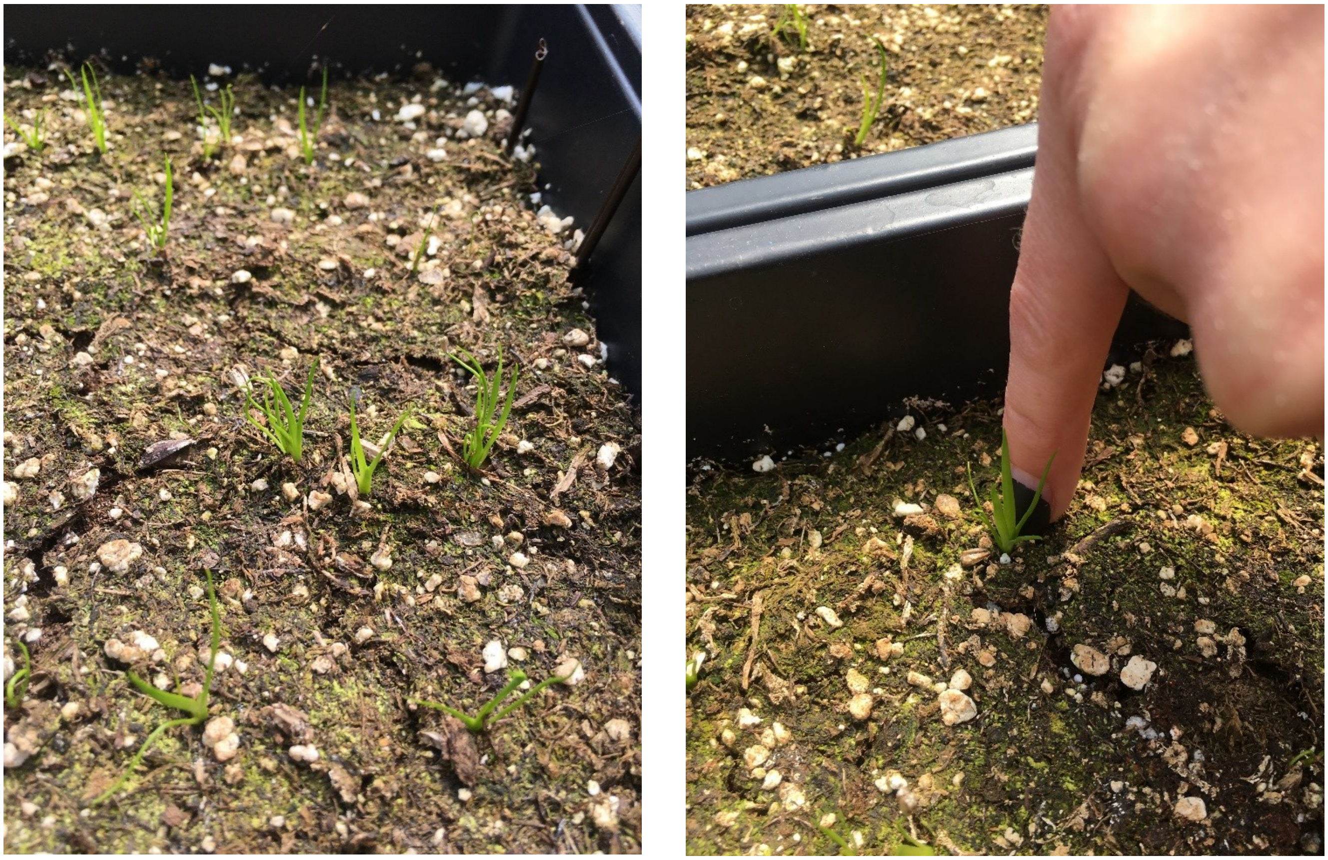

Figure 14. First growth of damp seeds recorded two months post-planting.

Figure 15. Dividing *A. calamus* into individual root segments, root washing and cold storage.

Figure 16. *Acorus calamus* with peat treatment, stored outside to replicate the weather condition similar to that of their natural counterparts.

Figure 17. Transplanting of all successful seedlings from rhizomes and seed trials.

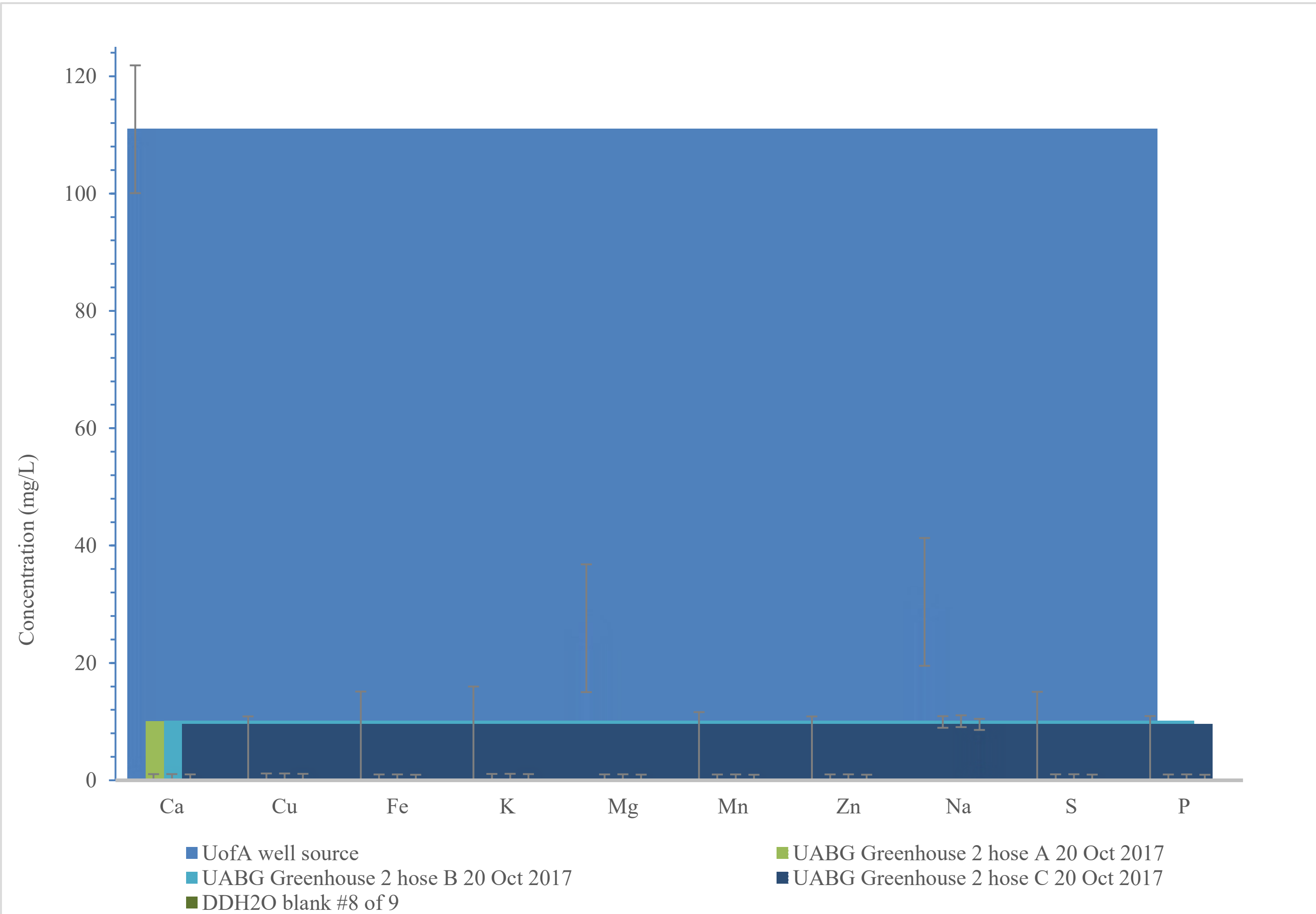

Figure 19. Vanadium and nickel concentrations in Hoagland's solution, mixed with water types at the University of Alberta Botanical Gardens.

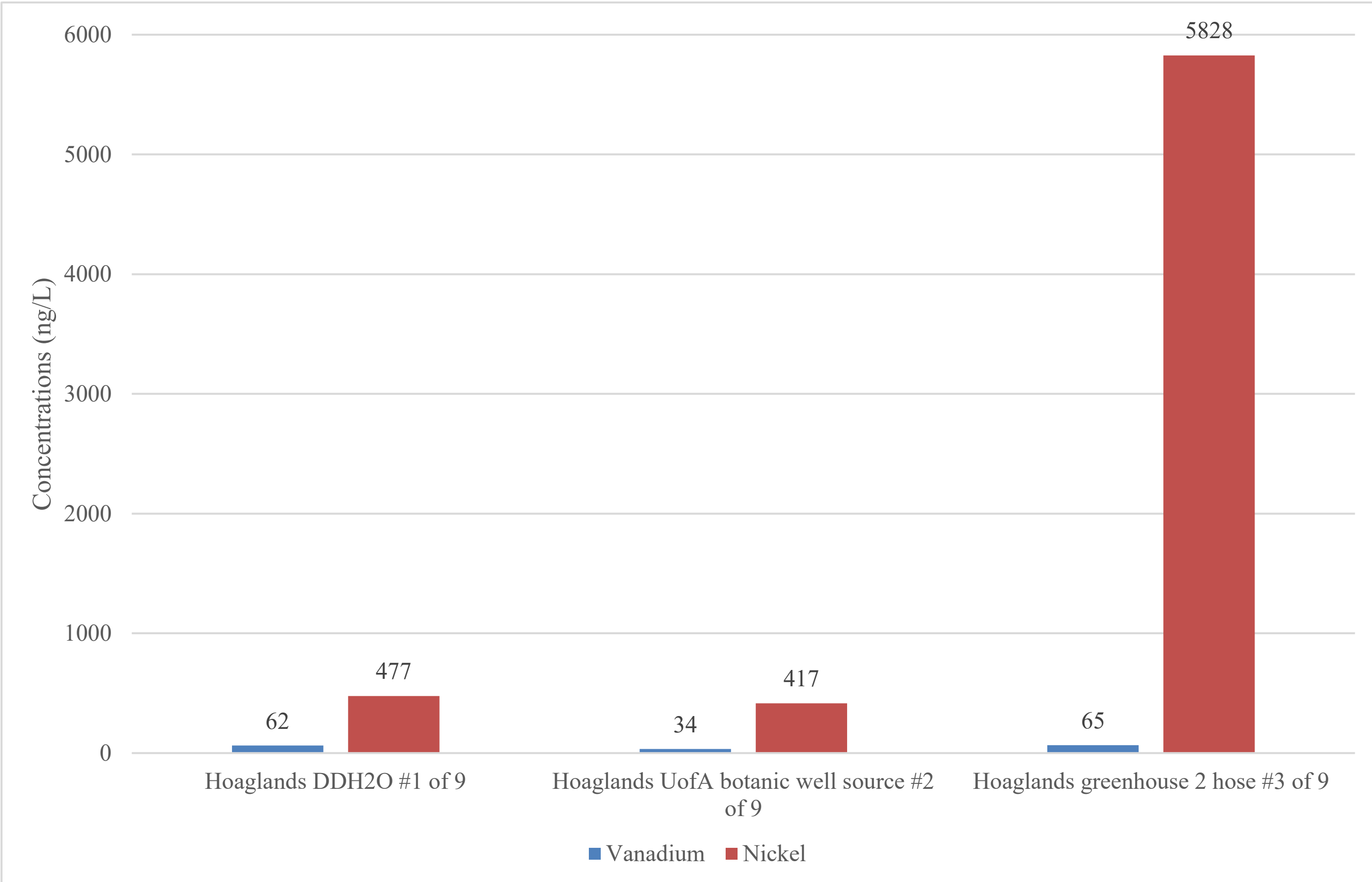

Figure 20. Chemical analysis of water from diverse sources.

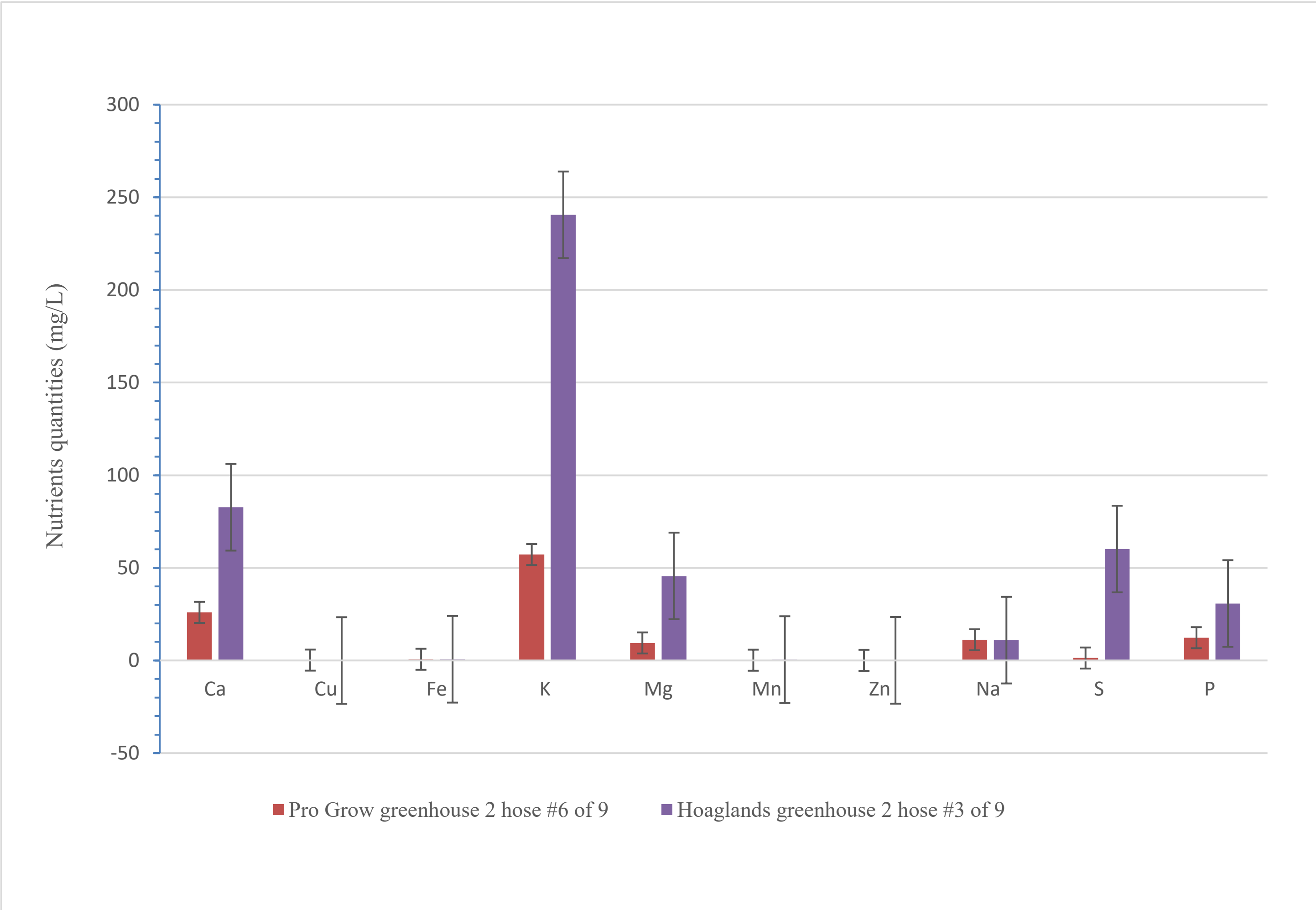

Figure 21. Chemical analysis of the two nutrient solutions selected for study.

Figure 22. Algal growth on plants during experiment. Plant containers were rinsed weekly to deter continued algal growth.

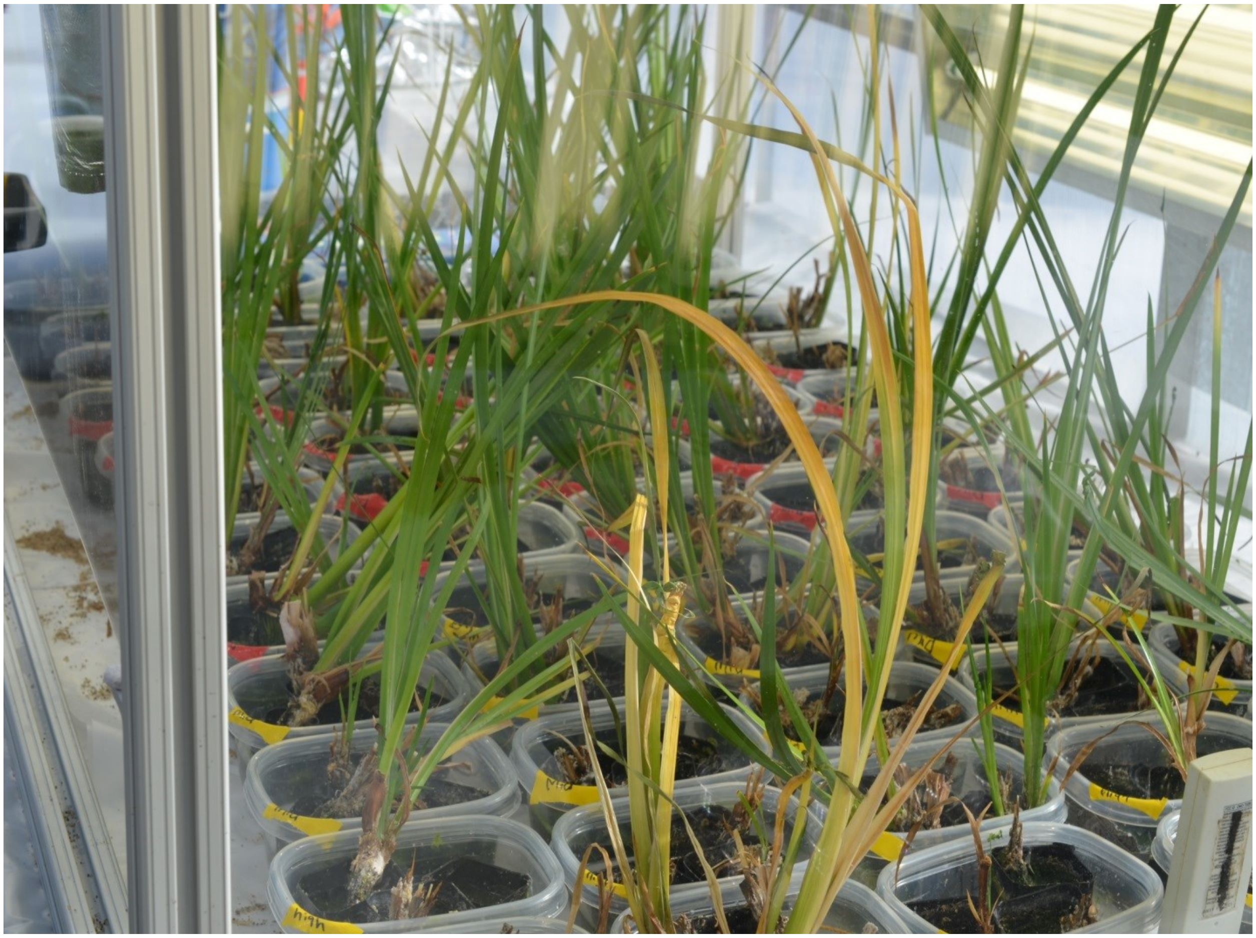

Figure 18. Potential signs of stress seen on high level vanadium treatment (0.10mg/L), shown by the yellowing of shoots.

Figure 24. Ceramic knife used to cut material during harvest. Pink interior of *A. calamus*

Figure 25. Drying of samples in an oven at 105 ^{0}C for 48 hrs.

Figure 26. Milling of samples employing agate jars and centrifugal ball mill.

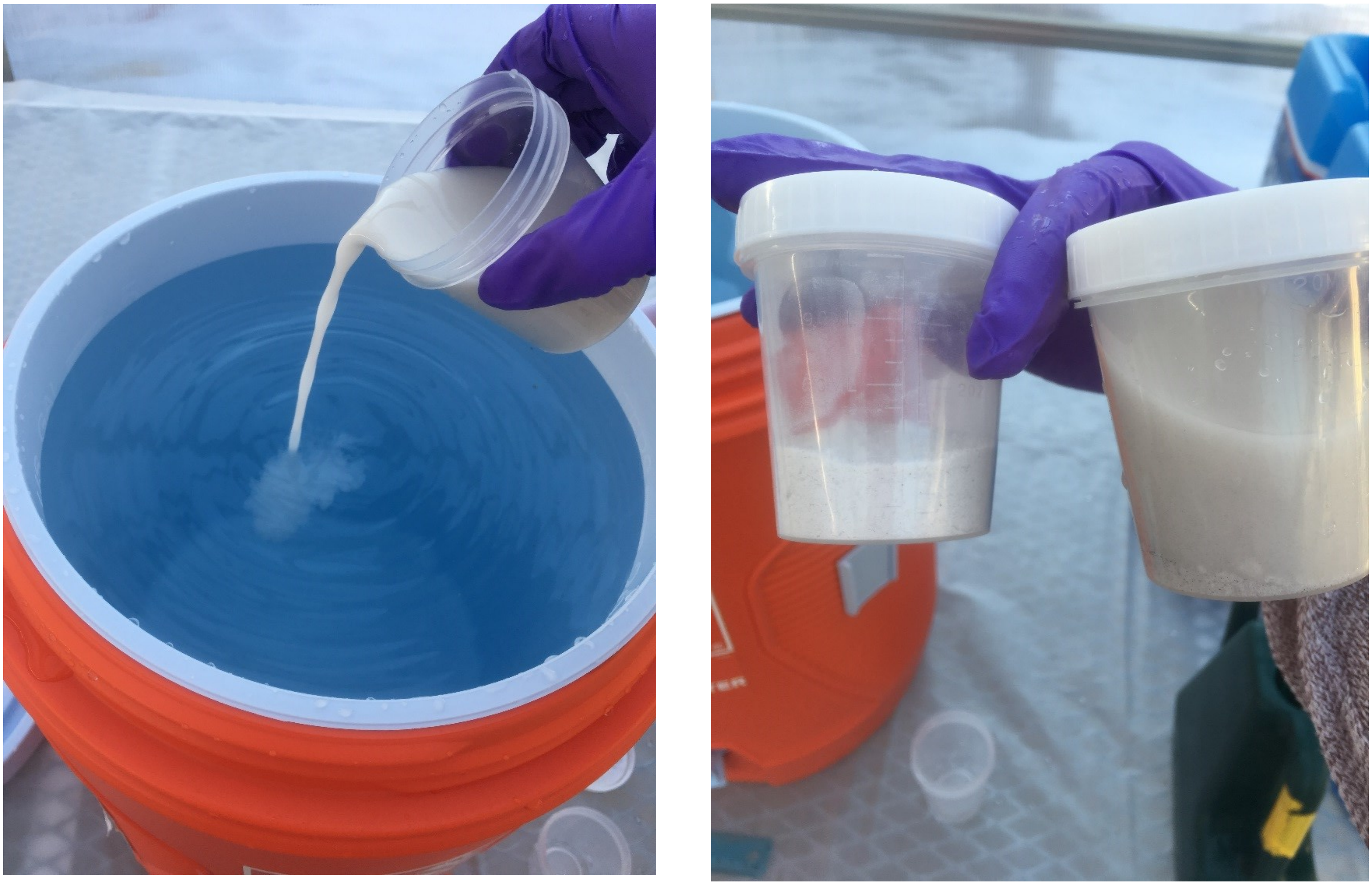

Figure 27. Addition of vanadium, nickel and nutrient slurries to 20L of water.

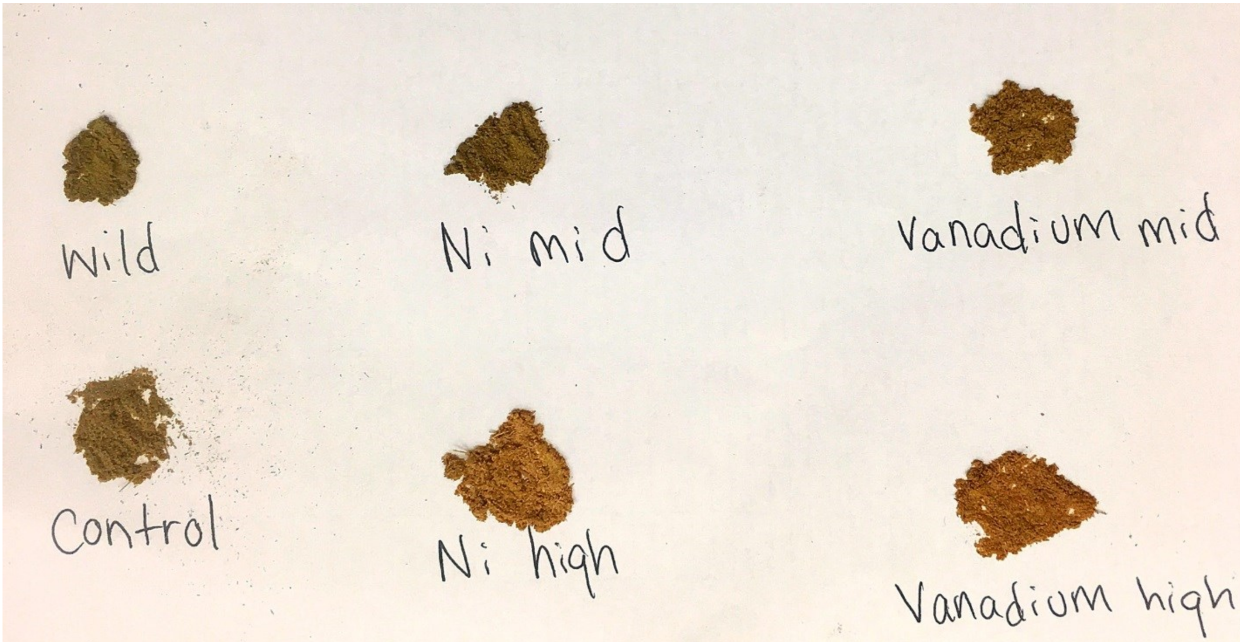

Figure 28. Wild plants ("wild"), nickel control ("control"), and nickel mid treatments ("Ni mid") were visibly green in colour. In contrast, vanadium mid ("vanadium mid"), vanadium high ("vanadium high"), and nickel high treatments ("Ni high") were visibly orange in colour.