

# Leonardite and Biochar for Mine Impacted Water and Soils

---

Technical Report prepared for Wapaw Bay  
Resources

By **Dr. Katherine Stewart and Dr. Amelie Janin,**  
**Yukon Research Centre**  
**November 2014**



## Contents

1. Executive Summary .....	3
2. Introduction.....	4
3. Methods .....	6
3.1 Metal adsorption capacity .....	7
3.2 Metal sequestration property.....	7
3.3 Pb leaching from hydrocarbon-contaminated soil .....	7
3.4 Plant growth and metal uptake.....	8
4. Results.....	9
4.1 Metal adsorption capacity .....	9
4.2 Metal sequestration in tailings .....	12
4.3 Pb leaching from hydrocarbon-contaminated soil .....	14
4.4 Plant growth.....	15
4.5 Plant metal uptake.....	17
5. Summary .....	20
6. Acknowledgments.....	21
7. References.....	21

## 1. Executive Summary

Immobilization of metals using soil amendment processes is increasingly being considered as an effective and low cost remediation alternative in the mining industry. Both leonardite, a carbon-rich material rich in humic acids and biochar, an organic material that has undergone pyrolysis, have shown to adsorb heavy metals, such as Cd, Zn and Pb and promote plant growth. We examined the potential to use leonardite and biochar for metal sequestration in mining impacted water and soils, by determining their capacity to adsorb metals in water, sequester metals in tailings, promote plant growth and prevent metal uptake in plants. Biochar removed up to 95% Cd, 90% Zn and 88% Pb from synthetic water and resulted in a 74% reduction of Cd and 18% of Zn leached from columns containing tailings. Whereas, leonardite adsorbed 93% Pb, but only 38% Cd and 29% Zn from synthetic water and resulted in column leachate with higher concentrations of Cd and Pb. Leonardite amendments caused decreases in pH and mobilization of Cd and Zn from tailings may be due to acidification. Above and belowground growth of two different northern native herb species (*Lupinus arcticus* and *Hedysarum alpinum*) in amended tailings were examined. Amendments had little influence on growth with only the leonardite and lime treatment showing increased belowground biomass. High levels of Cd, Zn and Pb were found in both plant species, however, it appears that biochar may help to reduce Cd and Zn bioavailability and leonardite may help to reduce Pb bioavailability. This initial trial demonstrates that both amendments show potential for on-going management of contaminated waters and tailings, however, additional liming agents are likely necessary with leonardite.

## 2. Introduction

In situ immobilization of metals using soil amendment processes is increasingly being considered as an effective and low cost remediation alternative (Mench et al. 2007, Kumpiene et al. 2008, Fellet et al. 2011). Leonardite is a carbon-rich material derived from the oxidation of Lignite and is rich in humic acid, whereas biochar is a product that results from the oxygen limited, pyrolysis of various biological ingredients, such as wood, fish or animal bone. Several studies have found biochar amendments result in significant decreases in the bioavailability of heavy metals associated with mining impacted soils (Namgay et al. 2006, Fellet et al. 2011, Beesley et al. 2010) and simultaneously improve physical, chemical and biological soil properties (Laird et al. 2010). Leonardite is known to improve soil conditions (Lao et al. 2005, Zeledón- Torunõ et al. 2005, Madejón et al. 2010) and has potential to significantly reduce metal bioavailability due to high metal adsorption capacity (Lao et al. 2005, Zeledón- Torunõ et al. 2005).

The study of solubility and bioavailability of metals in contaminated soils or water is important in remediation activities because they represent the most labile fractions subject to leaching and to being uptaken by plants and microorganisms (Adriano 2001). Both leonardite and biochar have shown to adsorb heavy metals, such as Cd, Zn and Pb from contaminated waters (Lao et al. 2005, Chen et al. 2011, Zeledón- Torunõ et al. 2005, Kolodynska et al. 2012, Regmi et al. 2012, Table 1). The removal of metals from both water and soils is highly pH dependent (Zeledón- Torunõ et al. 2005). While metal precipitation requires alkaline pH, metal adsorption is less pH dependent. Alteration of the pH by liming is a frequent remediation practice for trace element polluted systems (Adriano 2001, Madejón et al. 2009), however the effects of liming gradually reduce over time due to the dissolution and leaching of the liming agent (Ruttens et al. 2010). Biochars and leonardite are both highly recalcitrant and their effects may persist over long time periods (Steiner et al. 2007).

Table 1. Maximal adsorption capacity of biochar and leonardite for Cd, Cu, Pb, Ni and Zn found in previous studies. An asterisk (\*) indicates the study was completed with ‘activated’ biochar that was chemically enhanced prior to the metal adsorption experiment.

Metal	Max adsorption capacity (in mg metal/g adsorbent)		References
	Biochar	Leonardite	
<b>Cadmium</b>	16.6	50.6	Kolodynska et al 2012
	34*		Regmi et al 2012 Lao et al 2005
<b>Copper</b>	6.3	21	Kolodynska et al 2012
	31*		Regmi et al 2012 Toruno 2005
<b>Lead</b>	19.85	250.7	Lao et al 2005
<b>Nickel</b>		15.3	Zeledón-Torunõ 2005
<b>Zinc</b>	4.25		Kolodynska et al 2012

Phytostabilization of mine tailings is highly difficult, not only due to phytotoxic effects of elevated heavy metal concentrations, but also due to extreme pH values, low fertility, low water-holding capacity and unfavorable substrate structure (Fellet et al. 2011). In highly degraded soils amendments can not only assist in trace element stabilization, but may also directly impact plant cover establishment and the long-term improvement of soil quality (Pérez-de-Mora et al. 2005, Pérez-de-Mora et al. 2006). Both leonardite and biochar are known to promote seed germination and viability, increase plant biomass and the rate of root development (Chen and Aviad 1990, Nardia et al. 2002, Jones et al. 2012). In addition, these materials can help reduce heavy metal toxicity by reducing the bioavailability of contaminants, leading to improved revegetation of disturbed areas (Pérez-de-Mora et al. 2007).

We examined the potential to use leonardite and biochar for metal sequestration in mining impacted water and soils, by determining the capacity of leonardite and biochar to adsorb metals in water, sequester metals in tailings, promote plant growth and prevent metal uptake in plants (Figure 1).

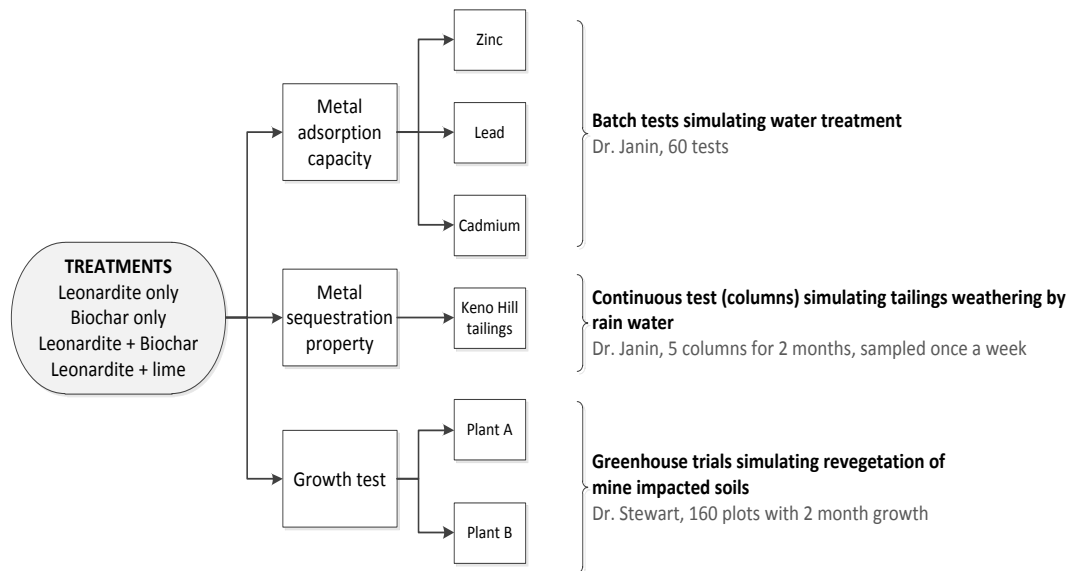


Figure 1. The objective of this research project is to examine the potential to use leonardite and biochar for metal sequestration in mining impacted water and soil. This was examined through examination of the capacity of leonardite and biochar to adsorb metals in water, the examination of the capacity of leonardite and biochar to sequester metals in tailings and exploration of the ability of leonardite and biochar to sequester metals and promote plant growth.

### 3. Methods

Our laboratory and greenhouse experiments used tailings and locally harvested seeds of native plant species from the Keno Hill Mining District (KHMD), which is one of the world’s highest-grade silver districts, located 330 km north of Whitehorse, Yukon, Canada. It is estimated that approximately 4,050,000 tonnes of tailings were deposited at a 130 ha site located in the McQuesten River Valley (63°55’26.4N, 135°29’76.1W). The tailings are highly variable with a pH ranging from 5.7 to 8.4 and texture varying from silt loam to sand. The tailings exceed the Canadian Council of Ministers of the Environment (CCME) industrial soil quality guidelines for allowable levels of Antimony (Sb), Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Silver (Ag), Titanium (Ti) and Zinc (Zn). A preliminary analysis of Cd, Zn and Pb in the tailings was performed. Tailings were digested using a HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> method (method USEPA 3050b) and analyzed by atomic adsorption spectrometry (PinAAcle™ 900 Atomic Adsorption Spectrometer, PerkinElmer, Ontario Canada). Tailings used in the metal sequestration and greenhouse experiments had approximate concentrations of Cd, Zn and Pb at 111 mg/kg, 739 mg/kg and 8374 mg/kg respectively.

The leonardite used in all experiments was provided by Wapaw Resources Inc. and was from their leonardite mine at Wapawekka Lake, Saskatchewan. The biochar used in all

experiments was provided by Diacarbon based in Burnaby British Columbia and was a wood (spruce, pine and fir mix) biochar.

### **3.1 Metal adsorption capacity**

Synthetic contaminated water for each heavy metal of interest (i.e. Cd, Zn, Pb) was created. The initial concentrations were 1.2 mg/L for the Cd solution, 0.68 mg/L for the Zn solution (initial targets of 1 mg/L) and 890 mg/L for the Pb solution (initial target of 1,000 mg/L). The pHs of solutions were adjusted using NaOH and H<sub>2</sub>SO<sub>4</sub> to a pH of 7. Leonardite or biochar at 2, 4, 6, 8, and 10g were mixed with the synthetic contaminated water resulting in solutions with leonardite or biochar ranging 10g/L to 50g/L. The leonardite or biochar solutions were then shaken at 180 rpm at room temperature for 24 hours. Water metal concentrations before and after exposure to leonardite or biochar were measured by atomic adsorption spectrometry (PinAAcle™ 900 Atomic Adsorption Spectrometer, PerkinElmer, Ontario Canada). The data was used to determine the adsorption capacity of each leonardite and biochar for Cd, Zn and Pb.

### **3.2 Metal sequestration property**

Tailings contaminated with Cd, Zn and Pb from KHMD were mixed with 4 treatments: i) Leonardite (6% v/v), ii) Biochar (6% v/v), iii) Leonardite and Biochar (each at 6% v/v) and iv) Leonardite (6% v/v) and dolomite lime (54.6% CaCO<sub>3</sub>, 41.5% MgCO<sub>3</sub> at 484 g/m<sup>2</sup>). The mixture was contained in PVC pipes (4 columns + 1 unamended control) each 7.4 L in size. Deionized water was pumped to the top of the columns and infiltration occurred at an average rate of 18.9 mL/hr. Samples were collected at the bottom of each column every 3-4 days and were then filtered through a 0.45µm filter, acidified, and analyzed for Pb, Cd, and Zn using atomic adsorption spectrometry. Contaminant concentration in column leachate was compared to assess the capacity of the biochar and leonardite to sequester metals leached from tailings.

### **3.3 Pb leaching from hydrocarbon-contaminated soil**

Due to the high metal adsorption capacity of leonardite detected for Pb, we included an additional trial to examine Pb adsorption on leonardite in a highly contaminated soil from the Waste Oil Pit (WOP) at the City of Whitehorse Landfill, Whitehorse, Yukon. The WOP soil had a cohesive tar-like texture and was contaminated with hydrocarbons in the F1, F2 and F3 fractions (323, 5000, 5000 mg/kg respectively), chlorinated compounds including Tetrachloroethylene (0.59 mg/kg) and Trichloroethylene (0.02 mg/kg) and As (16 mg/kg), Zn (780 mg/kg) and Pb (710 mg/kg). All of these contaminants exceeded the CCME limits for

commercial land use. Due to the cohesive texture of the soil, we also included a treatment with sand to potentially improve contact between the leonardite and soil through increasing the available surface area. Four treatments were included in the study: i) WOP (50 ml DI water, 50 g contaminated soil), ii) WOP + L (50 ml DI water, 42.5 g contaminated soil, 7.5 g Leonardite-2.5% by wt.), iii) WOP + S (75 ml DI water, 75 g contaminated soil, 50 g sand), iv) WOP + S + L (75 ml DI water, 67.5 g contaminated soil, 50 g sand, 7.5 Leonardite- 2.5% by wt). Each treatment was replicated 3 times and we measured Pb water concentrations prior to and following 24 hrs of shaking at 180 rpm by atomic adsorption spectrometry. The data was used to further assess the potential of leonardite to reduce Pb leaching from a hydrocarbon contaminated soil.

### 3.4 Plant growth and metal uptake

Tailings contaminated with Cd, Zn and Pb from KHMD were mixed with the same 4 treatments as those described in section 3.2. All treatments were also fertilized with a 19:19:19 fertilizer at a rate of 110 kg/ha to emulate fertilization practices currently being carried out at the mine site. Two northern native herb species (Arctic Lupin (*Lupinus arcticus*) and Alpine Sweetvetch (*Hedysarum alpinum*)) previously grown in Keno tailings were selected for the greenhouse trial (Stewart & Siciliano, 2014). There were 10 replicates (i.e. individual containers with two seedlings) for each treatment for each plant species. The greenhouse conditions and watering (6 ml deionized water (DI) per replicate every second day) were controlled to reflect typical summer growing conditions in the Keno area. Temperature was 11°C with no light from 22:00-4:00 and 16°C with 175  $\mu\text{mol}/\text{m}^2/\text{s}$  of light from 4:00-22:00. After two months of growth, above and belowground biomass of the herb species was examined across the 4 treatments. Plants were harvested divided into above and belowground components and thoroughly cleaned with DI water and small brushes to remove any tailings adhered to the exterior of the plant. All biomass was dried at 90°C for 36 hours before determining dry weights.

Following determination of biomass, above and belowground plant samples were digested using a nitric acid digestion (Zarcinas, 1987; Remon et al., 2013) and analyzed for Cd, Zn and Pb content using atomic adsorption spectrometry. Due to very low growth rates in the tailings *H. alpinum* plant samples were homogenized and pooled across treatments and then analysis was performed on 3 sub-samples per treatment. For *L. arcticus* 3 replicates from each treatment were randomly sampled and the entire replicate was digested and analyzed. Dry biomass used for digestions ranged from 25-36 mg. For quality assurance of plant analysis, we also digested and analyzed two certified reference plants (Spinach leaves-1570a and Peach leaves-1547, Standard Reference Materials, National Institute of Standards and Technology). Average recoveries were 92.9% and 99.6% for Cd and Zn respectively.



## 4. Results

### 4.1 Metal adsorption capacity

Both biochar and leonardite showed metal adsorption capacity. Up to 95% of Cd initially present in solution was removed by biochar after 24 hours of contact versus 38% by leonardite. With 10 g/L of leonardite or biochar, the maximal observed Cd loading was 49.2 mg Cd/g leonardite, which is closely comparable to the 50.6 mg/g adsorption capacity found by Lao et al (2005). The maximal loading observed for Cd on biochar was high at 110.6 mg Cd/g biochar (Figure 2), demonstrating that biochar was able to adsorb more than 10% of its mass in Cd. This high adsorption capacity of biochar for Cd has been found in other studies (Kolodynska et al 2012; Regmi et al 2012).

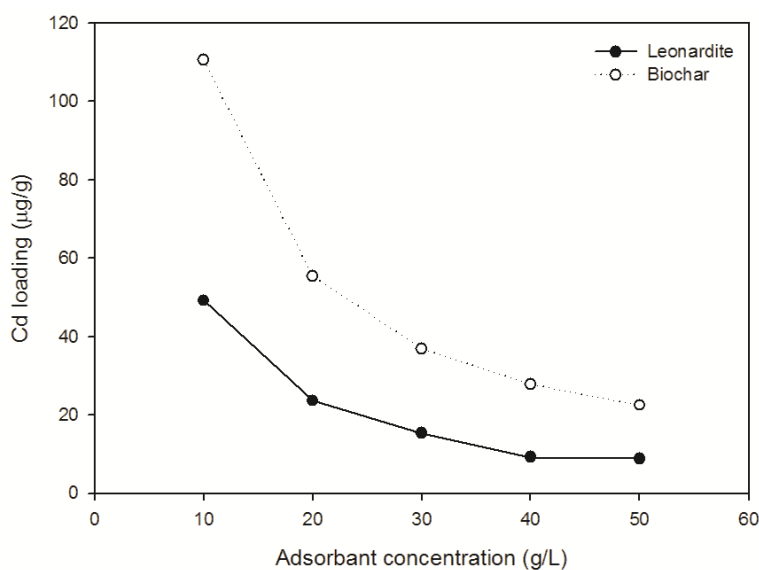


Figure 2. Cadmium loading on leonardite and biochar after 24 hrs with leonardite and biochar concentrations ranging from 10-50 g/L (Initial Cd concentration: 1.2 mg/L).

To our knowledge our study was one of the first to examine Zn adsorption on leonardite and we found a maximal adsorption capacity of 19.6 mg/g at 10g/L of leonardite (Figure 3). Biochar adsorption capacity was higher than leonardite; with a maximum of 51.9 mg/g at 10g biochar/L. Adsorption of Zn showed a similar trend to Cd adsorption with up to 90% of Zn removed by biochar after 24 hours of contact versus 29% by leonardite.

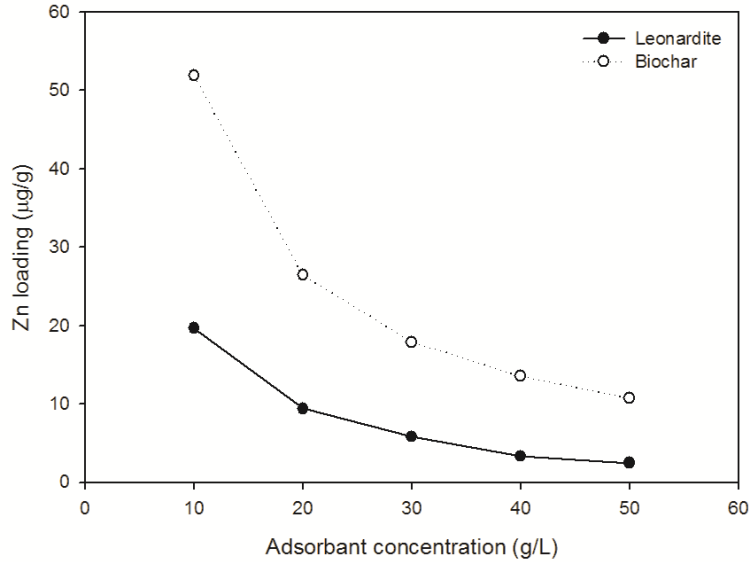


Figure 3. Zinc loading on leonardite and biochar after 24 hrs with leonardite and biochar concentrations ranging from 10-50 g/L (Initial Zn concentration: 0.68 mg/L).

We found that leonardite was capable of adsorbing up to 83.1 mg/g Pb (Figure 4). Although this is less than previously reported (250.7 mg Pb/g), leonardite exhibits a considerable adsorption capacity for Pb (Lao et al 2005). Leonardite demonstrated a high adsorption capacity for Pb, up to 8% of its mass in lead (83 mg/g), allowing this adsorbent to remove up to 93% Pb from the Pb-loaded synthetic water (initial concentration 890 mg/L) after 24 hours of contact with 10g/L leonardite. Unlike with Cd and Zn, biochar had a lower adsorption capacity for Pb, with a maximum loading of 22.2 mg Pb/g. At 10g biochar/L, only 26% of Pb was removed by biochar (Figures 2-4).

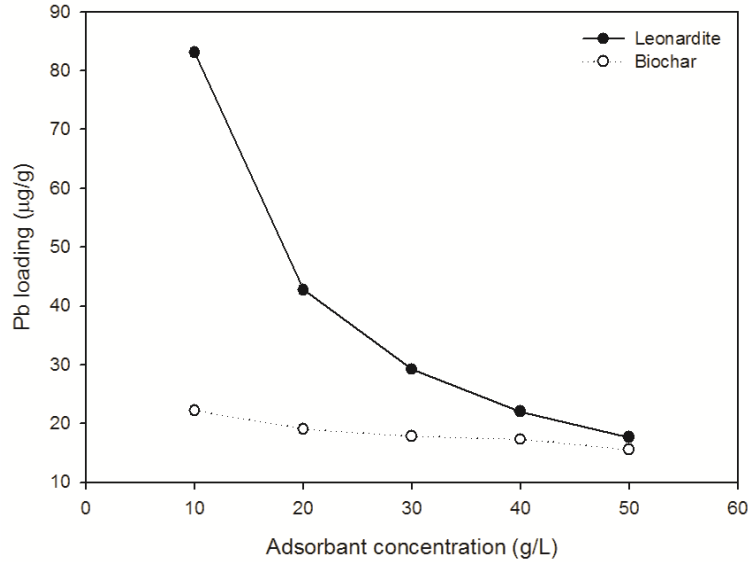


Figure 4. Lead loading on leonardite and biochar after 24 hrs with leonardite and biochar concentrations ranging from 10-50 g/L (Initial Pb concentration: 890 mg/L).

Solutions with an initial pH of 7 containing leonardite and biochar ranging in a solid to liquid ratio of 10-50% were held with 24 hours of contact between the water and each adsorbent. Both leonardite and biochar lowered the pH, however, leonardite lowered the pH to 2.9-3.4, while biochar lowered the pH less to 5.6-6.4 (Figure 5).

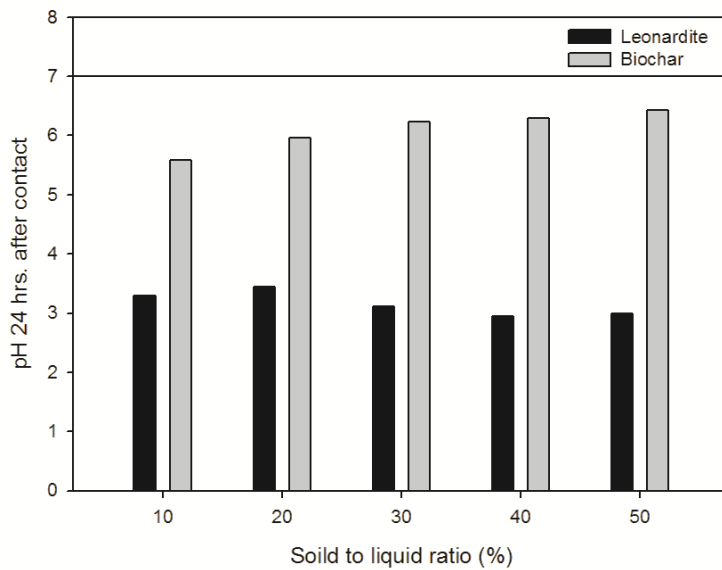


Figure 5. Change in pH after 24 hours of contact with leonardite or biochar with solid to liquid ratios ranging from 10 to 50%. Initial pH of 7 is shown as a reference line.

## 4.2 Metal sequestration in tailings

The results from our metal sequestration trial were consistent with the observations made in the metal adsorption experiment, with leonardite lowering the pH of the leachate filtering through the amended tailings columns. Columns amended with leonardite resulted in a leachate with a pH significantly lower than in the unamended control in the first month of column operation (Figure 6). Biochar had very little effect on the pH with leachate having a similar pH to the unamended control. Addition of lime in the leonardite-amended column did not raise the pH in the first month of operation. However, after 45 days, leachate from the column amended with lime and leonardite showed an increase in pH towards that of the unamended control.

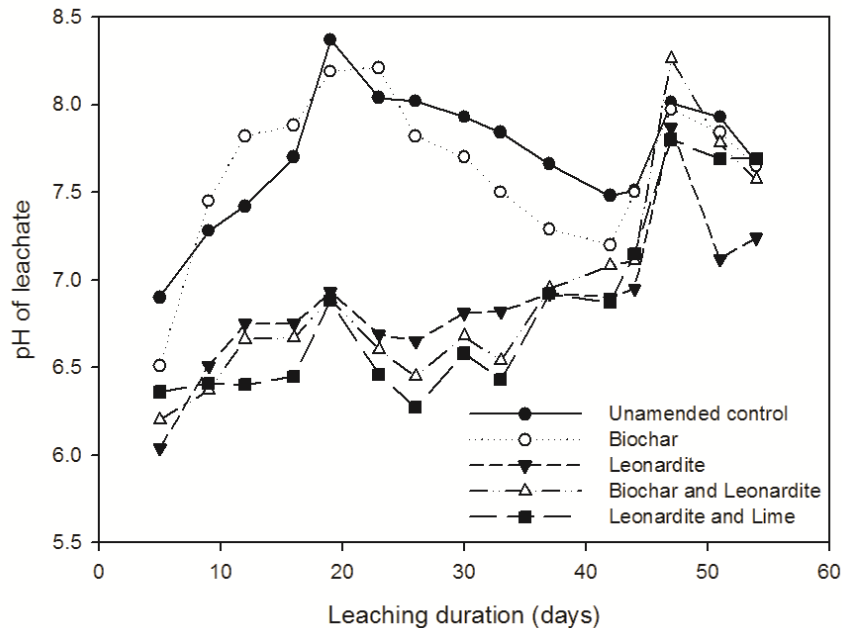


Figure 6. pH of leachate from tailings columns amended with biochar, leonardite, biochar and leonardite and leonardite and lime over 60 days.

Higher concentrations of Cd and Zn were leached out of the columns amended with leonardite in comparison to columns amended with biochar and the unamended column (Figure 7). These results suggest that Cd and Zn are mobilized from the tailings by the leonardite. Metals mobilization is likely caused by acidification and further dissolution of Cd and Zn salts, such as carbonate or hydroxide salts that are present in the tailings.

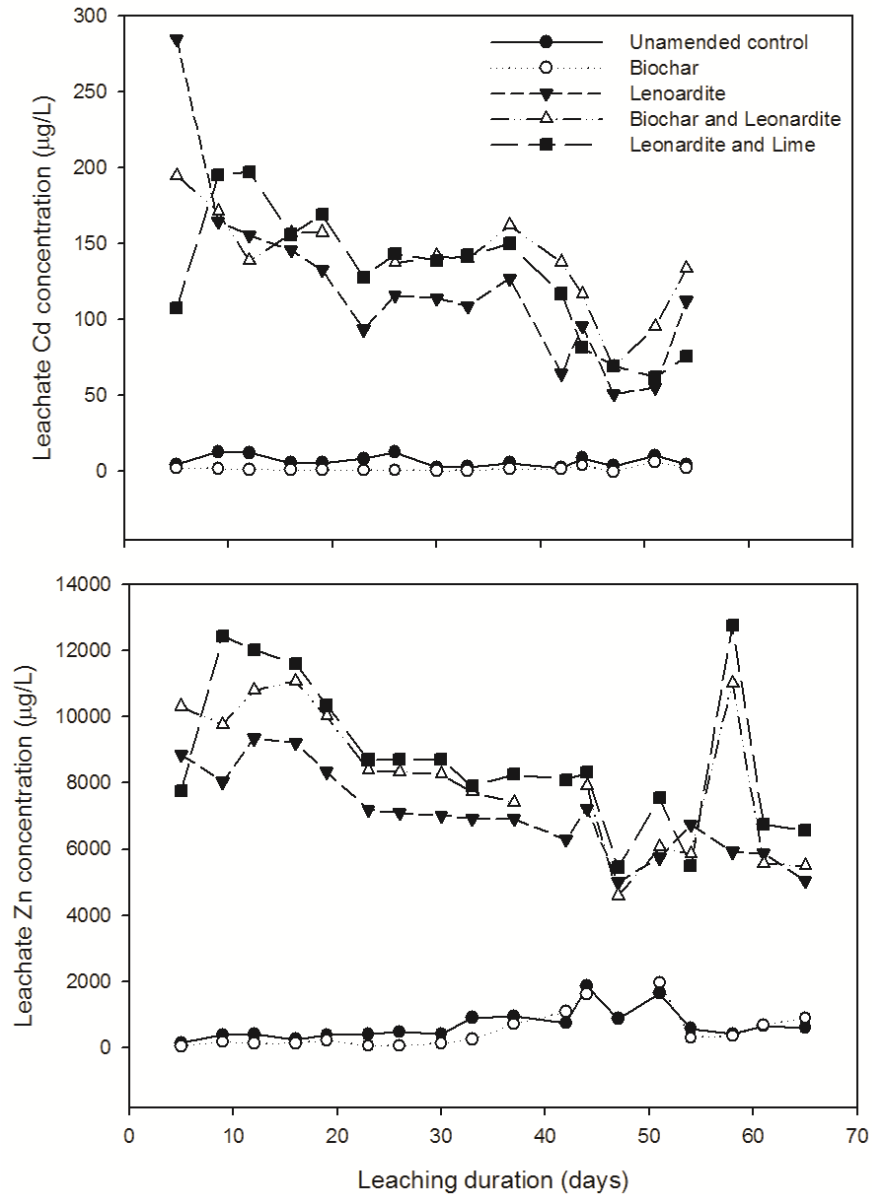


Figure 7. Cadmium and Zinc concentrations of leachate from tailings columns amended with biochar, lenoardite, biochar and lenoardite and lenoardite and lime over 65 days.

Leachate from the biochar-amended column had lower Cd and Zn concentrations than from the unamended column. Thus biochar appears to reduce the amount of Cd and Zn leached from the tailings. Lower Cd and Zn leaching may be explained by sorption of the metals at the surface of the biochar, as suggested by the results from the metal adsorption tests. Compared to the unamended control, leachate from the column with biochar had a 75.2 % reduction in Cd and a 26.0 % reduction in Zn (Table 2). Therefore, due to the high adsorption capacity of biochar for Cd and Zn it may prove to be a suitable amendment for metal sequestration in tailings, whereas

leonardite would not be suitable where Cd and Zn are contaminants of concern. We did not detect any Pb being leached from any of the columns during the experiment.

Table 2. Comparison of total Cd and Zn leached from unamended tailings columns and tailings columns amended with biochar over 70 days.

<b>Column Treatment</b>	<b>Total volume leached</b>	<b>Total Cd leached (ug)</b>	<b>Total Zn leached (ug)</b>
Unamended control	29.13	173.89	19,745
Biochar	28.04	43.12	14,620
% reduction	N/A	75.20	25.96

#### **4.3 Pb leaching from hydrocarbon-contaminated soil**

In the subsequent adsorption experiment with highly contaminated soils from the Waste Oil Pit (WOP) we found leonardite significantly reduced the Pb concentration in water following 24 hrs of shaking. The average Pb leached was reduced by 37.7% in treatments with leonardite and 56.9% in treatments with both leonardite and sand (Table 3). The WOP soil was very cohesive and had a tar-like consistency. The higher adsorption observed with sand is likely due to an increase in surface area of the contaminated soil resulting in better contact between the leonardite and Pb from the WOP soils. Knowing that previous studies have shown that leonardite might be effective at reducing hydrocarbons in soil (Moosley 1998) it is interesting to observe potential for Pb sequestration from a hydrocarbon contaminated soil. Soils contaminated with hydrocarbons frequently have Pb co-contamination, thus the use of leonardite for remediation of hydrocarbon-Pb contaminated soil could be highly valuable and should be further studied.

Table 3. Average lead concentrations found in water samples following 24 hrs of shaking of 50 g Waste Oil Pit contaminated soil in 50 ml of deionized water with 7.5g of leonardite (WOP + L) and without (WOP) and with 75 g of Waste Oil Pit contaminated soil and 50 g of sand in 75 ml of deionized water with 7.5g of leonardite (WOP + S + L) and without (WOP + S). The percent reduction in Pb concentration in water after 24 hrs is given.

<b>Treatment</b>	<b>Average Pb leached (mg Pb/g soil) <math>\pm</math> SD</b>
WOP	0.61 $\pm$ 0.16
WOP + L	0.38 $\pm$ 0.05
% reduction	37.70
WOP + S	1.02 $\pm$ 0.07
WOP + S + L	0.44 $\pm$ 0.03
% reduction	56.86

#### 4.4 Plant growth

Both northern native herb species showed poor growth after two months in the greenhouse with higher belowground biomass than aboveground biomass. We did not detect any influence of the treatments on the below and aboveground biomass accumulation for *L. arcticus*, but found significantly higher belowground biomass for *H. alpinum* grown in tailings amended with leonardite and lime (Figure 8). Leonardite is known to stimulate root growth with both increased root length and development of secondary roots (Chen and Aviad 1990) and can reduce soil pH around roots helping to convert unavailable nutrients to plant accessible forms (Vaughan and Donald, 1976). As observed in the metal sequestration trial, pH did increase over time with lime and the higher belowground biomass for *H. alpinum* in the leonardite and lime treatment may reflect a positive growth response to the increased pH, as well as, the presence of leonardite. Pérez-de-Mora et al. 2010 similarly found that repetitive amendment of contaminated soils with leonardite and lime had a positive effect on biomass accumulation.

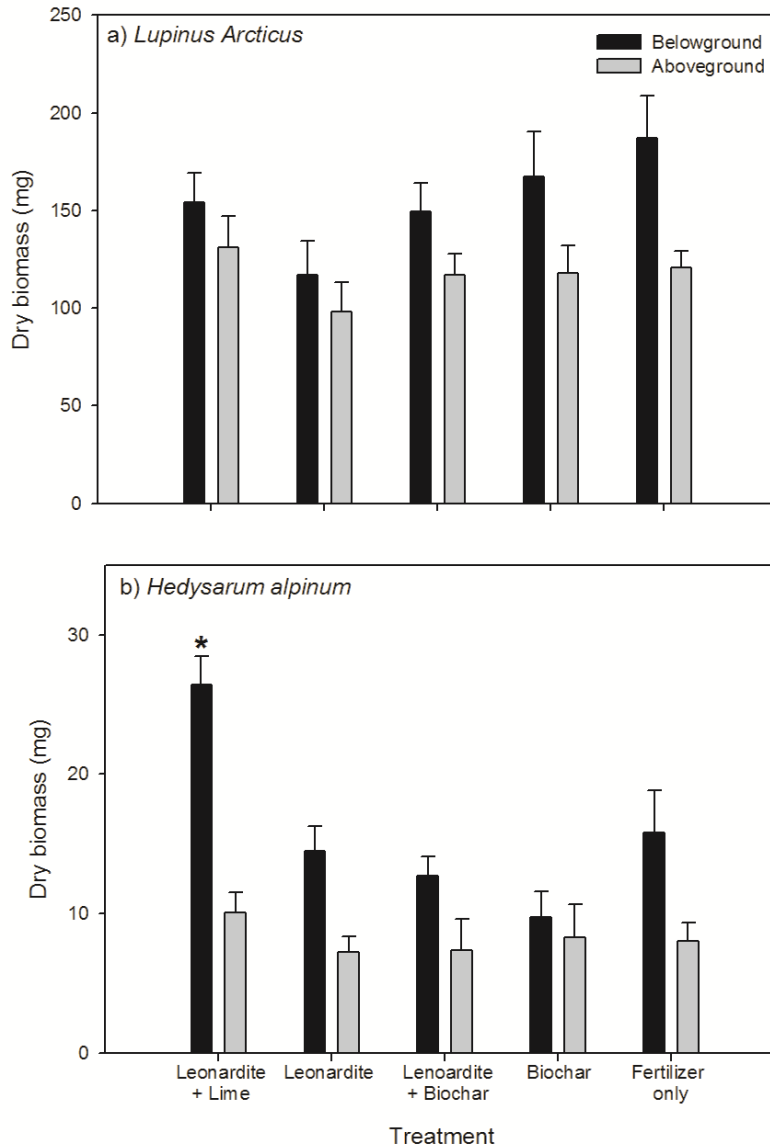


Figure 8. Belowground and aboveground biomass of *Lupinus Arcticus* (Arctic Lupin) and *Hedysarum alpinum* (Alpine Sweetvetch) following two months of growth in a greenhouse in tailings treated with leonardite and lime, leonardite, leonardite and biochar, biochar and fertilizer only. Bars are means with standard error. There were no significant differences in above or belowground biomass with treatment for *L. arcticus* or aboveground biomass for *H. alpinum* (ANOVA,  $p > 0.05$  for all comparisons). Belowground biomass of *H. alpinum* was significantly higher in tailings treated with leonardite and lime compared with all other treatments (ANOVA, TukeyHSD,  $p < 0.01$  for all comparisons).



#### 4.5 Plant metal uptake

Both northern native herb species showed signs of heavy metal toxicity with chlorosis and a purpling and reddening of leaves after approximately 30 days of growth in the tailings (Figure 9). After two months of growth stem death was evident for some replicates of both herbs. Depressed plant growth, chlorosis and a reddening of younger leaves are common symptoms of Zn toxicity (Kabata-Pendias and Pendias 1992; CCME, 1999; Reichmann, 2002). Pb is a nonessential element to plants and adverse effects are generally only seen at relatively high Pb concentrations (Pahlsson 1989; CCME, 1999). Plants experiencing Pb toxicity can have smaller leaves, chlorotic and reddish leaves with necrosis, short black roots and stunted growth (Pahlsson 1989; CCME, 1999).

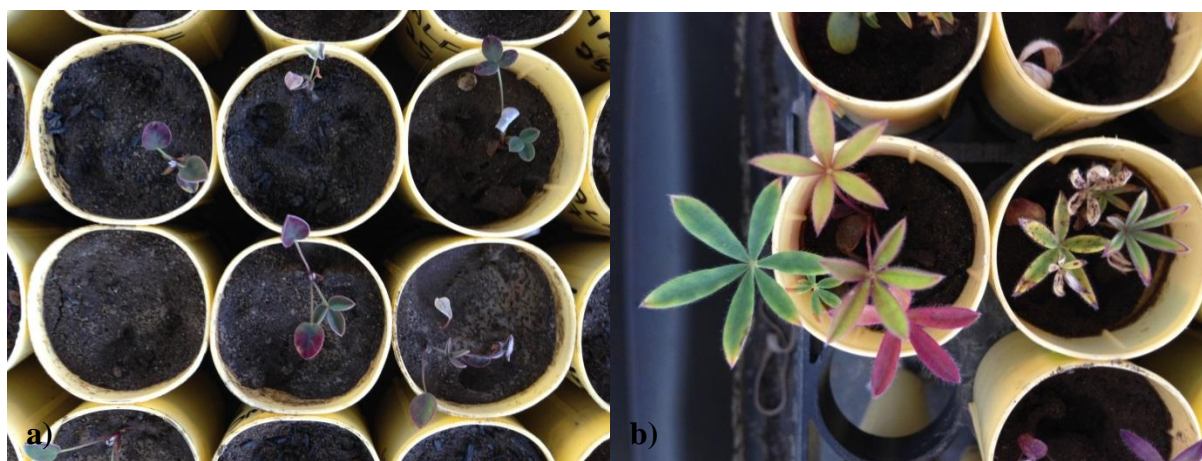


Figure 9. *Hedysarum alpinum* (a) and *Lupinus arcticus* (b) showing signs of chlorosis (i.e. yellowing and purpling of leaves) after approximately 30 days of growth in tailings likely due to heavy metal toxicity.

In addition to our observations we also detected metal concentrations in plant biomass of the two native plants that indicated high levels of toxicity. While *L. arcticus* had higher average concentrations of Cd and Zn, *H. alpinum* had higher average concentrations of Pb (Figure 10). Uptake of Cd varies in plants depending on species, while some plants show translocation others do not, with Cd accumulation occurring in the leaves of some and in the roots of others (Kim et al. 1988; Boon and Soltanpour 1992; CCME, 1999). We found higher Cd concentrations in the belowground biomass of both species; however *L. arcticus* had a much higher average Cd concentration (119 mg/kg) than *H. alpinum* (43 mg/kg). The lowest soil Cd concentrations at which phytotoxic effects in plants have been observed are 2.5 to 4 mg/kg. The average Zn concentration in *L. arcticus* was 1506 mg/kg and in *H. alpinum* 911 mg/kg and phytotoxic

effects have been found in plants where concentrations in soil Zn are as low as 50 mg/kg (Sheppard et al., 1993; CCME, 1999). Reduction in biomass has been observed in plants grown in soils with Pb concentrations as low as 50 mg/kg (CCME, 1999). *L. arcticus* biomass had an average concentration of 242 mg Pb/kg and *H. alpinum* biomass an average concentration of 485 mg Pb/kg.

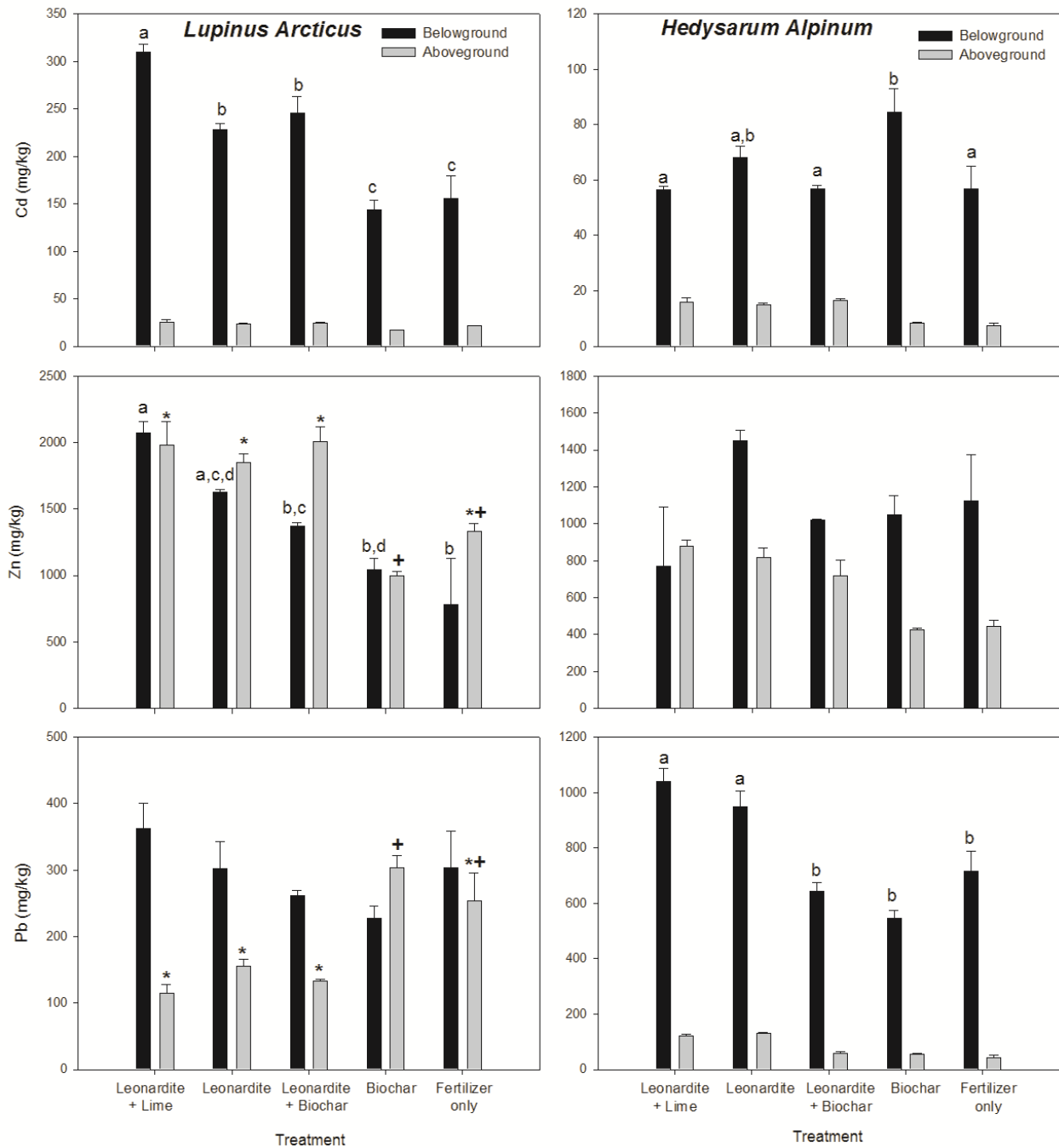


Figure 10. Concentrations of Cd, Zn and Pb found in the belowground and aboveground biomass of *Lupinus arcticus* (left) and *Hedysarum alpinum* (right) after two months of growth in tailings with leonardite and biochar amendments. Significant differences in Cd, Zn and Pb concentrations in belowground biomass between treatments (\*+) and Cd, Zn and Pb concentrations in aboveground biomass between treatments (a-d) are shown for each plant species. Different symbols or letters indicate significantly different mean metal concentrations between treatments (ANOVA, TukeyHSD,  $p < 0.05$  for all comparisons).

Overall metal concentrations tended to be higher for Cd and Pb in belowground biomass than aboveground biomass, however, metal concentrations in belowground biomass were species dependent with much higher concentrations of Cd in *L. arcticus* belowground biomass and much higher concentrations of Pb in *H. alpinum* belowground biomass.

There were clear treatment differences in metal uptake for each of the northern native plant species (Figure 10). For *L. arcticus* we observed higher Cd in belowground biomass and Zn in above and belowground biomass in tailings amended with leonardite, but lower concentrations of Pb in aboveground biomass. For *H. alpinum* there was no difference in Zn concentrations with treatment and Pb was higher in belowground biomass in treatments with leonardite. It appears that biochar may help to reduce the bioavailability of Cd and Zn and leonardite may help reduce the bioavailability of Pb, however, neither biochar or leonardite treatments were significantly different than the unamended controls respectively.

We observed strong species and treatment interactions that suggest that different native northern plants may respond differently to the use of soil amendments for mine site restoration. For example, while *L. arcticus* showed a reduction in Pb uptake with leonardite treatments, *H. alpinum* showed the opposing trend. It should be noted that due to the limited growth, particularly of *H. alpinum*, we had to pool across replicates and our biomass samples for digestions and subsequent analysis were very small (i.e. 25 mg). Therefore, the metal uptake results for *H. alpinum* should be interpreted with caution due to the minimal dry biomass used for metals analysis. Overall, metal uptake in plants, particularly of *L. arcticus*, were reflective of our adsorption and metal sequestration results, with biochar showing a strong adsorption capacity for Cd and Zn, thereby reducing its bioavailability and uptake and leonardite showing a strong adsorption capacity of Pb thereby reducing its bioavailability and uptake. \

## 5. Summary

Both biochar and leonardite demonstrated metal adsorption of Cd, Zn and Pb. However, adsorption rates for biochar were considerably higher for synthetic water with Cd and Zn and concentrations of Cd and Zn in leachate from tailings with biochar were reduced compared to the unamended control. Adsorption rates for leonardite were considerably higher in synthetic water with Pb and concentrations of Pb were significantly reduced in a hydrocarbon-Pb contaminated soil with leonardite. Leonardite amended tailings resulted in the mobilization of Cd and Zn from the tailings, which was likely due to acidification of the water flowing within the tailings. We also observed that both biochar and leonardite lowered pH of mining contaminated water and tailings. However, leonardite lowered the pH to a much greater extent, which likely accounts for the potential acidification and subsequent mobilization of Cd and Zn from leonardite amended tailings columns.

The poor growth of both northern native herb species on the tailings is most likely due to heavy metal toxicity. Other studies that have examined vegetation establishment of the UKHM tailings have also found chlorosis in species such as, *Arctostaphylos uva-ursi*, *Carex aquatilis*, and *Equisetum arvense* (Clark & Hutchinson, 2005). High levels of Cd, Zn and Pb were found in both plant species, however, it appears that biochar may help to reduce Cd and Zn bioavailability and leonardite may help to reduce Pb bioavailability. We did not however see a significant difference in metal uptake for either amendment in comparison to the unamended control. However, the increased belowground biomass for *H. alpinum* in the leonardite and lime treatment suggests that these amendments may still provide positive growth effects.

In the context of remediation and phytostabilization of mine tailings decreases in pH, particularly those observed with leonardite amendments, are undesirable and a liming agent may need to be considered. In the tailings amended with both leonardite and lime, pH appeared to equilibrate after 45 days and further studies are needed to examine the longer-term influence of a combined leonardite and lime treatment. Biochar shows strong potential for metal removal in both mining impacted water and tailings. This initial trial demonstrates that both leonardite and biochar have potential as amendment technologies for remediation and restoration in northern Canada.

## 6. Acknowledgments

We would like to thank Raymond Potié of Wapaw Bay Resources for his collaboration on this project. We would also like to thank Hiromi, Isobel Ness, Annie-Claude Letendre, Justine Viros, Carrie Boles and Patrick Soprovich for laboratory and greenhouse assistance. The project was funded by an NSERC Applied Research and Development Grant.

## 7. References

Adriano, D.C. 2001. Trace Elements in Terrestrial Environments: Biogeochem, Bioavailability and Risks of Metals, 2nd ed., Springer, New York.

Beesley, L., Marmiroli, M. 2011. The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. *Environ Pollut*, 159:474-480.

Boon, D.Y., Soltanpour, P.N. 1992. Lead, cadmium and zinc contamination of Aspen garden soils and vegetation. *J Environ Qual* 21:82-86.

Canadian Council of Ministers of the Environment. 1999. Canadian soil quality guidelines for the protection of environmental and human health: Cadmium (1999). In: Canadian

environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Canadian Council of Ministers of the Environment. 1999. Canadian soil quality guidelines for the protection of environmental and human health: Lead (1999). In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Canadian Council of Ministers of the Environment. 1999. Canadian soil quality guidelines for the protection of environmental and human health: Zinc (1999). In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chen, Y., Aviad, T. 1990. Effects of humic substances on plant growth. In: P. MacCarthy, C.E. Clapp, R.L. Malcolm and P.R. Bloom (Eds.) Humic Substances in Soil and Crop Sciences: Selected Reading, p. 161-186. American Society of Agronomy, Madison, WI.

Chen, X., Chen, G., Chen, L., Chen, Y., Lehmann, J., McBride, M.B., Hay, A.G. 2011. Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresource Technol* 102:8877-8884.

Clark, A., and Hutchinson, T. 2005. Enhancing natural succession on Yukon mine tailings sites: A low-input management approach. MPERG Report 2005-3. Whitehorse, Yukon.

Fellet, G., Marchiol, L., Delle Vedove, G., Peressotti, A. 2011. Application of biochar on mine tailings: Effects and perspectives for land reclamation. *Chemosphere*, 83:1262-1267.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., and Murphy, D.V. 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biol Biochem* 45:113-124.

Kabata-Pendias, A., and H. Pendias. 1992. Trace elements in soils and plants. 2d ed. CRC Press. Boca Raton, FL.

Kolodynska, D., Wnetrzak, R., Leahy, J.J., Hayes, M.H.B., Kwapinski, W., Hubicki, Z. 2012. Kinetic and adsorptive characterization of biochar in metal ions removal. *J Chem Eng* 197:295-305.

Regmi, P., Moscoso, J.L.G., Kumar, S., Cao, X., Mao, J., Schafran, G. 2012. *J Environ Manage* 109:61-69.

Kim, S.J., Chang, A.C., Page, A.L., Warneke, J.E. 1988. Relative concentrations of cadmium and zinc in tissue of selected food plants grown on sludge-treated soils. *J Environ Qual* 17:568-573.

Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments – A review. *Waste Manage* 28:215–225.

Laird, D.A., Fleming, P., Davis, D.D., Horton, R., Wang, B., Karlen, D.L. 2010. Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma*, 158:443-449.

Lao, C., Zeledon, Z., Gamisans, X., Sole, M. 2005. Sorption of Cd(II) and Pb(II) from aqueous solutions by a low-rank coal (leonardite). *Sep Purif Technol* 45: 79-85.

Madejón, E., Pérez-de-Mora, A., Burgos, P., Cabrera, F., Lepp, N.W., and Madejón, P. 2010. Do amended, polluted soils require re-treatment for sustainable risk reduction? — Evidence from field experiments. *Geoderma* 159:174-181.

Madejón, E., Madejón, P., Burgos, P., Pérez-de-Mora, A., Cabrera, F., 2009. Trace elements, pH and organic matter evolution in contaminated soils under assisted natural remediation: A 4-year field study. *J Hazard Mat* 162:931-938.

Mench, M., Vangronsveld, J., Lepp, N.W., Ruttens, A., Bleeker, P., Geebelen, W., 2007. Use of soil amendments to attenuate trace element exposure: sustainability, side effects, and failures. In: Hamon, R.E., McLaughlin, M., Lombi, E. (Eds.), *Natural Attenuation of Trace Element Availability in Soils*. SETAC Press, Pensacola, Florida, pp. 197–228.

Moosley, R. 1998. The effect of humates on remediation of hydrocarbon and salt contaminated soils. In: *Proceedings from the 5<sup>th</sup> International Petroleum Environmental Conference*, Albuquerque, New Mexico, October 20-23, 1998.

Namgay, T., Singh, B., Singh, B.P. 2006. Plant availability of arsenic and cadmium as influenced by biochar application to soil. *World Congress of Soil Science: Soil Solutions for a Changing World*. August 1-6, 2010, Brisbane Australia.

Nardia, S., Pizzeghello, D., Muscolob, A., Vianello, A. 2002. Physiological effects of humic substances on higher plants. *Soil Biol Biochem* 34:1527-1536.

Pahlsson, A.B. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water Air Soil Pollut.* 47:287–319.

Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaekel, P., and Schloter, M., 2006. Microbial structure and function in a heavy metal contaminated soil: effects of plant growth and different amendments. *Soil Biol Biochem* 38:327–341.

Pérez-de-Mora, A., Madrid, F., Cabrera, F., and Madejón, E. 2007. Amendments and plant cover influence on trace element pools in a contaminated soil. *Geoderma* 139:1-10.

Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Madejón, E. 2010. Implications of repetitive amendment additions for in situ phytostabilization of trace elements in contaminated soils. Report no. HM01B-1, accessed November 6, 2014, <http://digital.csic.es/bitstream/10261/42805/1/Implications%20of%20repetitive%20amendment.pdf>

Pérez-de-Mora, A., Ortega-Calvo, J.J., Cabrera, F., Madejón, E., 2005. Changes in enzyme activities and microbial biomass after “in situ” remediation of a heavy metal-contaminated soil. *Appl Soil Ecol* 28:125–137.

Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Madejón, E. 2010. Implications of repetitive amendment additions for in situ phytostabilization of trace elements in contaminated soils. Report no. HM01B-1, accessed November 6, 2014, <http://digital.csic.es/bitstream/10261/42805/1/Implications%20of%20repetitive%20amendment.pdf>

Reichmann, S. 2002. The responses of plants to metal toxicity: A review focusing on Copper, Manganese and Zinc. The Australian Minerals and Energy Environment Foundation, Occasional Paper No. 14 ISBN 1-876205-13-X

Remon, E., Bouchardon, J.-L., Le Guédard, M., Bessoule, J.-J., Conord, C., Faure, O. 2013. Are plants useful as accumulation indicators of metal bioavailability? *Environmental Pollution* 175: 1-7.

Ruttens, A., Adriansen, K., Meers, E., De Vocht, A., Gebelen, W., Carleer, R., Mench, M., Vangrosveld, J., 2010. Long-term sustainability of metal immobilization by soil amendments: cyclonic ashes versus lime addition. *Environ Pollut* 158:1428–1434.

Sheppard, S.C., W.G. Evenden, S.A. Abboud, and M. Stephenson. 1993. A plant life-cycle bioassay for contaminated soil, with comparison to other bioassays: Mercury and zinc. *Arch. Environ. Contam. Toxicol.* 25:27–35.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., Macedo, J.L.V.D., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil* 291:275–290.

Stewart, K.J. and Siciliano, S.D. 2014. Potential contribution of native herbs and biological soil crusts to restoration of the biogeochemical nitrogen cycle in mining impacted sites in northern Canada. *Restoration Ecology*. *Accepted September 2014*.



Vaughan, D., MacDonald, I.R. 1976. Some effects of humic acid on cation uptake by parenchyma tissue. *Soil Biol. Biochem.* 8:415-421.

Zarcinas, B.A., Cartwright, B., Spouncer, L.R. (1987) Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry, *Communications in Soil Science and Plant Analysis* 18:1, 131-146.

Zeledón-Torunõ, Z., Lao-Luque, C., Solé-Sardans, M. 2005. Nickel and copper removal from aqueous solution by an immature coal (leonardite): Effect of pH, contact time and water hardness. *J Chem Technol Biotechnol* 80:649-656.