Potential for using paper mill fly-ash as an alternate liming material and mobility and leachability of heavy metals in fly-ash and biochar amended agricultural soil in Western Newfoundland

by

GNANAKARAN MAHESWARAN

A thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science

Boreal Ecosystems and Agricultural Sciences
School of Science and the Environment
Grenfell Campus
Memorial University of Newfoundland

December 2019

St. John's Newfoundland and Labrador

Abstract

Most agricultural soils in Western Newfoundland are acidic and need lime to raise the soil pH to be productive. Corner Brook Pulp and Paper Ltd produces a substantial amount of fly-ash which is being disposed at a local landfill. This study was conducted to assess the potential for using fly-ash as a liming material for an agricultural soil (pH:5.5) in Western Newfoundland with the addition of biochar for heavy metal stabilization. Heavy metal concentration in the soil and fly-ash were analysed and compared with soil and compost guidelines. Lab scale leaching and pot experiments were conducted to assess the leaching and bioavailability of heavy metals in fly-ash amended soil with different biochar rates. As per quality guidelines, only part of the lime requirement can be substituted by fly-ash. Biochar increased the soil pH and biomass production. Total heavy metal leached from biochar treated soils were low and unlikely to cause groundwater contamination. In general biochar reduces the leachability and the bio availability of heavy metals. Application of biochar could provide a sustainable solution for the heavy metal stabilization of fly-ash treated soil.

Acknowledgments

First and foremost, my sincere gratitude to my advisor Dr. Mano Krishnapillai, for the guidance, encouragement and support given during laboratory work as well in the successful completion of this research. I am very grateful for his knowledge, patience and unerring ability to keep me on the right track. I would like to express my special thanks to my co-advisor Dr. Doreen Churchill for sharing her broad knowledge and for the valuable comments and discussions. My special thanks to advisory committee member Dr. Lakshman Galagedara, who have provided valuable comments, suggestions, discussions and encouragement when most needed, and inputs to my research work.

I would like to express my special thanks to forestry and Wildlife Research Division (formerly the Centre for Forest Science and Innovation) under the Department of Fisheries and Land Resources, Government of Newfoundland and Labrador and Research Office of the Grenfell Campus, Memorial University of Newfoundland for their financial support.

I would like to express my special thanks to Corner Brook Pulp and Paper Ltd for their support and providing required ash samples. Also, I would like to thank Dr. Xiaobin Guo for his help with field visit and soil sample collection. I express my warm thanks to Dr. Tao Yuan and Jamie Warren for their assistance in the laboratory and my special thanks to the staff of Department of Facilities management, Grenfell campus for their help with fabricating the experimental units. I especially thank my friends Chameera Illawathure, Kamaleswaran Sadatcharam and Gayantha Sashika Perara who have helped in various aspects of this research.

I wish to offer my heartfelt thanks to all my family: parents, brother and sisters, especially my loving wife, for their support and encouragements.

All your support and cooperation are much appreciated.

Table of Contents

List of Tables

Table of Figures

List of Appendices

List of Abbreviations and Symbols

AOAC	Association of Official Analytical Chemists
BD	Bulk density
CEC	Cation exchange capacity
cm	Centimeter
EC	Electrical conductivity
CBPPL	Corner Brook Pulp and Paper Ltd
CCME	Canadian Council of Ministers of the Environment
CFIA	Canadian Food Inspection Agency
ha	Hectare
kg	Kilogram
Max	Maximum
Min	Minimum
n	Number of samples
NL	Newfoundland and Labrador
Mg	Megagram
mg	Milligram
C	Carbon
N	Nitrogen
Ca	Calcium
P	Phosphorus
K	Potassium
Mg	Magnesium
S	Sulfur
Fe	Iron
Mn	Manganese
Zn	Zinc
Cu	Copper
Co	Cobalt
B	Boron
Mo	Molybdenum

Cd	Cadmium
Ni	Nickel
Pb	Lead
V	Vanadium
Cr	Chromium
As	Arsenic
Se	Selenium
Hg	Mercury
mm	Millimetre
PBRS	Pynn's Brook Research Station
ASTM	American Society for Testing and Materials
CCE	Calcium Carbonate Equivalent
USDA	United States Department of Agriculture
OM	Organic matter
ICP-MS	Inductively coupled plasma mass spectrometry
h	Hour
mL	Milliliter
DOC	dissolved organic carbon
DW	Dry weight
N	North
W	West

Co-authorship Statement

Chapter 1 “Fly-ash from Pulp and Paper Mill: A Potential Liming material for agricultural soils in Western Newfoundland” and chapter 2 “Effect of Biochar on leaching and bioavailability of heavy metal on fly-ash amended soil” have been in preparation for submission to Journal (Gnanakaran, M., Krishnapillai, M, Churchill, D and Galagedara, L., 2019). Gnanakaran Maheswaran, the thesis author was the primary author and Dr. Mano Krishnapillai (supervisor), was the corresponding and the fourth author. Dr. Churchill (co-supervisor) and Dr. Galagedara (committee member) were second and third authors, respectively. All authors were part of the same research project which was led by Dr. Mano Krishnapillai. For the work in Chapter 1 and Chapter 2, the overall research strategy was developed by Dr. Mano Krishnapillai with input from all members of the group. Mr. Gnanakaran Maheswaran was responsible for the specific methodology, data collection, analysis, and interpretation and writing of the manuscript. Dr. Churchill and Dr. Galagedara provided inputs for the experiment, data interpretation, and manuscript editing. Dr. Mano Krishnapillai as the project lead and the main supervisor provided research plans and guidance for the entire process.

CHAPTER 1: Fly-ash from Pulp and Paper Mill: A Potential Liming Material for Agricultural Soil in Western Newfoundland

1.1 Background

Most agricultural soils in the Western Newfoundland are strongly leached under natural conditions and they are acidic with a loamy texture (Acton and Gregorich, 1995). These acidic soils need a large amount of lime per hectare to achieve the desired pH level for cultivation and regular applications are required to maintain the soil pH in a desirable range for crop production (Atlantic Soil Fertility Committee, 1970). There is a significant cost for farmers due to this high lime requirement. Failure to add lime to acidic soils reduces crop yields (Schwartz and Follett, 1979) wasting much of the farmers' fertilizer investment and increasing the cost of production.

Figure 1.1: The aerial view of Corner Brook Pulp and Paper Ltd

The use of wood biomass as a fuel source for the Pulp and Paper industry has led to the production of wood ash as a by-product (Poykio et al., 2004). Presently, Corner Brook Pulp and Paper Ltd (CBPPL) (**Figure 1.1 and 1.2**) mill uses a hog fuel (bark, wood, construction wood waste and saw mill waste) and waste oil mix as a fuel source for their boiler which generates steam for paper manufacturing and energy production. The fuel mixture is composed of approximately 90% biomass (wood waste and sludge from waste treatment) and 10% of waste oil (Production manager, CBPPL). Due to incinerating a large amount of biomass, a considerable amount of total ash is produced in the boiler.

Annually, considerable amounts of fly-ash and bottom ash, are generated from mill operation and disposed to landfills (Department of Environment and Climate Change, 2017). CBPPL mill is currently collecting 10,000 to 15,000 Mg of fly-ash each year. Ash is disposed at the local landfill site with an approximate annual cost of $250,000 (Churchill and Kirby, 2010). This material must be handled properly to prevent a serious environmental threat since leachate from wood ash may contain a high concentration of heavy metals and other contaminants (Poykio et al., 2004). There is a high potential for wood ash to be used as an alternative liming material and fertilizer in agricultural lands (Demeyer et al., 2001) due to its high pH (Campbell, 1990; Pitman, 2006) and thus the ability to increase the pH of acidic soils resulting in improved nutrient uptake by crop plants (Naylor and Schmidt, 1989; Williams et al., 1996).

Figure 1.2: The side view of Corner Brook Pulp and Paper Ltd

In addition, there is a demand for low-cost liming materials in the Atlantic region due to high soil acidity and the high cost of liming materials (Atlantic Soil Fertility Committee, 1970).

There are various advantages to diverting the ash generated by the pulp and paper industry as an alternative liming agent, not only would it save on disposal fees, but there would be environmental benefits like reduction of landfill disposal, reducing the risk of groundwater contamination etc. In addition, farmers can save money due to the increase in fertilizer efficiency and cost reduction for liming material due to the available ash (Alberta Agriculture, 2002). Some studies have revealed that wood ash from paper mill would have some metal contaminants (Pokio et al., 2004; Pitman, 2006; Jukic et al., 2017), which can have long-term negative effects on the ecosystem (Singh et al., 2011) when applied to agricultural soils as a liming material. That may limit the use of ash as a substitute for agricultural liming material.

1.2 Effect of application of fly-ash in agriculture

1.2.1 Improvement in soil properties

Soil properties as influenced by fly-ash application have been studied by several researchers for utilizing this industrial waste as a soil amendment. The change in physical and chemical properties of soil due to application of fly-ash amendment vary according to the original properties of soil and fly-ash but certain generalization could be made in most situation. Use of fly-ash applications may improve different soil properties for crop production (Demeyer et al., 2001;Sahu et al., 2018)

1.2.2 Physical properties

The application of fly-ash in agriculture may be helpful in improving physical properties of soil hence fertility and crop yield to a significant level (Rautaray et al., 2003). It modifies the structure of soil (Sahu et al., 2018) such that it reduces bulk density, increases porosity, aeration workability, root penetration and cation exchange capacity (CEC) which increase water and nutrients holding capacity of soil (Chang et al., 1977; Wong and Wong, 1990). This improvement in water holding capacity is beneficial for the growth of plants, especially under rainfed agriculture (Sahu et al., 2018). Fly-ash retards the nutrient loss with leaching water. Due to its fine particle nature (Demeyer et al., 2001), it has a high surface area which aids in retaining maximum nutrient in the soil. Because of the dominance of silt-sized particles in flyash, this material is often substituted for topsoil in surface-mined lands, thereby improving the physical condition of the soil, especially water holding capacity (Adriano and Weber, 2001; Jala and Goyal, 2006).

1.2.3 Chemical properties

Fly-ash has immense potential as a soil-ameliorating agent in agriculture, forestry and wasteland reclamation because of its characteristics. Various studies have shown that application of wood ash increases soil pH and decreases the exchangeable Al content of acid soils (Lerner and Utzinger, 1986; Ohno and Erich, 1990; Unger and Fernandez, 1990; Huang et al., 1992; Ulery et al., 1993; Kahl et al., 1996; Krejsl and Scanlon, 1996; Meiwes, 1995; Muse and Mitchell, 1995; Naylor and Schmidt, 1989; Williams et al., 1996). Several studies have shown that fly-ash reacts quicker with soils than lime, resulting in a rapid increase in pH, but only for a relatively short period (Clapham and Zibilske, 1992; Muse and Mitchell, 1995). The application of fly-ash to the soil will undoubtedly increase the concentration of major cations and anions in the soil solution (Kahl et al., 1996; Williams et al., 1996). Wood ash contains large amounts of micronutrients and soil amendment with wood ash will increase their concentration in the soil solution. One of the major influences of fly-ash on soil nutrient availability is to increase soil pH (Yunusa et al., 2012).

1.2.4 Biological properties

There are several studies done regarding the effects of fly-ash amendment on soil biological properties. Increased microbial activity was reported for ash-amended soils containing sewage sludge (Pitchel, 1990). Presence of organic matter has an additive effect as it reduces the concentration of toxic metals through sorption, lowers the C/N ratio and provides organic compounds. The application of lignite fly-ash reduced the growth of seven soil borne plant pathogenic microorganisms (Karpagavalli and Ramabadran, 1997). The application of fly-ash up to 15 Mg ha/year increase the activity of enzymes such as invertase, amylase, dehydrogenase and protease but enzyme activity decreased with higher levels of fly-ash application (Sarangi et al., 2001).

1.2.5 Fly-ash as a source of plant nutrients

Fly-ash is a direct source of nutrients and contains essential macronutrients including phosphorus (P), calcium (Ca) potassium (K), Magnesium (Mg) and Sulfur (S) and micronutrients like iron (Fe), manganese **(**Mn), zinc (Zn), copper (Cu), cobalt (Co), boron (B) and molybdenum (Mo). Some are rich in heavy metals such as cadmium (Cd) and nickel (Ni) (Unger and Fernandez, 1990; Ohno and Erich, 1990; Ohno, 1992; Meiwes, 1995; Kahl et al., 1996; Williams et al., 1996). The concentrations of major elements in fly-ash are highly variable (Demeyer et al., 2001). Boron is a good example of an important micronutrient that is readily supplied by fly-ash (Yunusa et al., 2012). Use of fly-ash along with chemical fertilizers and organic materials in an integrated way can save the amount of chemical fertilizer used as well as increase the fertilizer use efficiency while reducing the cost of production.

1.2.6 Use of fly-ash in composting

Some researchers have shown that fly-ash can be used to make a quality compost with different organic materials. A compost product from a 1:1 volume ratio of sludge and fly-ash was easily spread, containing many essential nutrients for plant growth as well as the liming ability (Hackett et al., 1999). Fly-ash was co-composted with wheat straw and 2% rock phosphate (w/w) for 90 days and fly-ash addition at 20% level resulted in the lowest C/N and highest available and total phosphorus. Increasing the addition of fly ash from 40 to 60% (w/w) did not exert any detrimental effect on either C:N or the microbial population (Sahu et al., 2018).

1.2.7 Improvement in growth, yield and nutrient quality of crops

Several field and greenhouse experiments confirmed that plants benefited and improved their growth from the nutrients contained in fly-ash (Basu et al., 2009). Under accountable management plans, land application of fly-ash can benefit agricultural production and improve the nutrient quality of crops by providing supplemental levels of some important elements (Patterson, 2001). The concentrations of trace elements (Fe, Mn, Zn and Cu) in plants decrease often with the application of wood ash, due to lower availability of these elements at higher soil pH (Clapham and Zibilske, 1992; Krejsl and Scanlon, 1996; Sahu et al., 2018) and it improves the quality of the crops. Ca and K contents of plants increase noticeably with the application of fly-ash (Demeyer et al., 2001) and it increased the plant growth. Concentrations of K, S, B, and Zn were found to be greater in plant tissue samples collected from fly-ash amended soils compared to the control for both barley and canola (Patterson, 2001).

1.2.8 Disease control

The application of fly-ash was found to have nematicide effect (Sahu et al., 2018) and was recommended for the management of root-knot disease in tomato caused by *Meloidogyne sp*. and to provide nutrients (Ahmad and Alam, 1997; Khan et al., 1997). Also tomato cultivars grown on fly-ash amended soils had a higher tolerance to wilt fungus (Basu et al., 2009; Sahu et al., 2018; Khan & Singh, 2001). The application of fly-ash reduced the growth of seven soil-borne pathogenic microorganisms (Karpagavalli and Ramabadran 1997). More than 50 species of insect pests of various major crops were susceptible to fly-ash treatment (Basu et al., 2009).

1.2.9 Improvement of degraded/marginal land

Mining has resulted in hectares of land which are physically, nutritionally and biologically poor in nature (Shrivastava and Kumar, 2015). These lands are characterized by poor water-holding capacity, infertility, high acidity or salinity of the soil (Brown et al., 2003). The solution for these degraded lands is the establishment of an economically feasible and permanent cover of vegetation (Shrivastava and Kumar, 2015). But the natural succession on these lands takes longer as low pH (Brown et al., 2003) is the major negative factor in natural revegetation of Newfoundland soil. Improving the productive capacity of degraded soils is particularly important for rapid rehabilitation (Brown et al., 2003) to support sustainable development. Fly-ash can be used as a potential soil amendment for degraded soils to improve soil properties in different ways to support the reclamation efforts by the mining industry and government agencies.

1.3 Objective of this study

1.3.1 General objective

The general objective of this study is to assess the potential of fly-ash from CBPPL as an alternate liming material for Newfoundland agricultural soil.

1.3.2 Specific objectives

- To evaluate the quality of soil and fly-ash by comparing with different soil and compost quality guidelines
- To determine the lime requirement and the application rate of the agricultural soil
- To determine the maximum allowable application rate of fly-ash to the soil under study based on the heavy metal contents and the guidelines.

1.4 Materials and Methodology

1.4.1 Soil analysis

The required soil for this study was sampled from land area in the Pynn's Brook Research Station (PBRS), Pasadena, NL, Canada. The site is located (49°04'23"N, 57°33'39"W) in the Humber Valley Watershed in the western part of the island of Newfoundland (**Figure 1.3**). The soil samples were collected from shallow depths (0-30 cm) to represent the majority of root zone in humid regions.

Figure 1.3: Soil sample collection in the field

The collected soil sample was air-dried for one week and sieved to a particle diameter of <2 mm. Particle size analysis done by using the hydrometer method (Kroetsch and Wang, 2007) showed that the soil has a sandy loam texture. Different physicochemical parameters of the soil were analyzed using standard methods. Standard methods and instruments used to measure different soil properties are shown in **Table 1.1**.

Table 1.1. Soil property measured, instrument used and the standard method

Soil property	Instruments	Standard method
Soil Texture	Standard hydrometer (ASTM, USA)	Hydrometer method (Kroetsch and Wang, 2007)
Bulk Density (g/cm^3)	Core sampler with a sliding hammer	Core sample method (Hao et al., 2007)
Soil Moisture Content (%)	Convection oven (Thermo Scientific, USA)	Gravimetric with oven drying (Topp et al., 2007)
pH_{Cacl2}	portable pH meter	0.01 M $CaCl_2$ method (Hendershot et al., 2007)
ECw (mS/cm)	EC/TDS/Temperature meter	1:2, soil: deionized water (Miller and Curtin, 2007)

Heavy metal concentration in the soil sample was analyzed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after acid digestion (EPA method 3050B). For determination of total heavy metal content of the soil samples, 1 g of each soil sample (oven dried at 70°C for 48 h) was accurately weighed into a digestion tube and 10 mL concentrated nitric acid: water, 1:1 v/v added. The sample was then heated at 95°C on a heating block for 15 min without boiling. After cooling at room temperature for 5 min, 5 mL concentrated HNO_3 was added and the sample was heated at 95°C for 30 min. Additional 5 mL aliquots of concentrated HNO_3 was added until no brown fumes were given off. The solution was then allowed to evaporate to <5 ml. After cooling, 2 mL of water and 3 mL of 30% H_2O_2 were added and heated until effervescence subsided, and the solution cooled. Additional H_2O_2 was added until effervescence ceased (but no more than 10 ml H_2O_2 was added). This stage was continued for 2 h at a temperature less than boiling point. Then, the solution was allowed to evaporate to <5 ml. After cooling, the sample was filtered through Whatman No. 41 filter paper into a 50 mL volumetric flask, and then made up to the mark with distilled water.

The heavy metal content data was compared with Canadian Council of Ministers of the Environment (CCME) Soil Quality Guidelines. The soil sample was analyzed for lime application requirement for two different type of forages at Soil & Plant Laboratory, St. John’s, Government of Newfoundland and Labrador.

1.4.2 Fly-ash sample collection and analysis

The composite sample of fly-ash was collected from CBPPL mill during the month of October 2017. Collected fly-ash sample (n=12) was analyzed for different physicochemical parameters. Heavy metal contents (Vanadium (V), chromium (Cr), arsenic (As), selenium (Se), mercury (Hg), Ni, Cu, Zn, Cd, Co and Pb) of the fly-ash sample were analyzed by by undergoing acid digestion followed by analysis using ICP-MS using the same method (EPA method 3050B) as used for the soil analysis. The heavy metal contents were compared with different quality guidelines. Calcium Carbonate Equivalency (CCE) of fly-ash sample was calculated by using Titrimetric Method.

A 1 g sample of dried fly-ash and a 50 mL portion of 0.5 mol dm^{-3} HCl were added to a 250 mL Erlenmeyer flask, and the sample was boiled gently for 5 min. The sample was cooled and filtered under gravity using filter-paper. The filtrate was titrated with 1.0 mol dm^{-3} NaOH to a permanent, faint pink endpoint using phenolphthalein as indicator then, the percent calcium carbonate was calculated.

1.4.2.1 Calculation of fly-ash requirement rate

The lime requirement was converted to an ash recommendation by the following calculation (Lickacz, 2002).

Equation 1 Ash recommendation

$$\text{The rate of ash required} = \frac{100}{CCE\ of\ ash\ (A)} \times \frac{100}{100 - \ moisture\ \%\ (B)} \times lime\ requirement\ (C)$$

Where:

- **A** = CCE of the fly-ash. CCE is a measure of the liming ability of the ash compared to pure calcium carbonate
- **B** = per cent moisture in the fly-ash.
- **C** = lime requirement as provided by the soil testing laboratory.

1.4.3 Statistical analysis

Statistical analyses to compare the average results were performed using a one sample t test. The Statistical analyses were carried out by using SPSS version 2010.

1.5 Results and Discussion

1.5.1 Soil analysis

The soil type was an Orthic Humo-Ferric Podzolic soil and the soil texture in the top 0–30 cm soil layer was sandy loam with sand 66.3% (± 3.2), silt 25.2% (± 4.6), and clay 8.5% (± 1.3), according to the United States Department of Agriculture (USDA) soil classification. pH, soil Organic Matter (OM) (%), Bulk Density (BD) (g/cm^3) and CEC (cmol/kg) of analysed soil samples were 5.5, 3.82 ,1.3 and 15.2, respectively.

Table 1.2 shows the different physicochemical parameters and the available nutrient content of the studied soil.

Table 1.2. Physicochemical characteristics of soil

pH $CaCl_2$	CEC cmol/kg	OM %	BD g/cm^3	Available Nutrients %				Texture		
				Mg	Ca	K	P	Sand %	Silt %	Clay %
5.5	15.2	3.82	1.3	0.27	1.1	0.12	0.03	66.3	25.2	8.5

Soil pH affects the physical, chemical, and biological properties of soils and crop yields. Also, it plays a significant role in the solubility of nutrients and metals within the soil profile. The results indicate that the pH of the soil is very low (5.5) and strongly acidic. In strongly acidic soils, the availability of the macronutrients as well as micronutrients such as molybdenum and boron are reduced (Lohry, 2007). Soil sample analysis show that available nutrient content is very low (**Table 1.2).** At this pH level, certain micronutrient deficiencies are common on some sandy soils.

Low pH in topsoil may affect microbial activity, most notably decreasing legume nodulation (Bargaz et al., 2018). This soil needs a lime application to increase the soil pH to a desired level for effective crop production.

CEC is also very important in crop productivity. Soil pH also influences CEC of soil colloids that have pH-dependent type of charge (Karak et al., 2005). The results indicate that the soil studied in this study has a low CEC. Soils with a low CEC are more likely to develop deficiencies in potassium and magnesium (Lombin, 1979). According to the result, the studied soil has a relatively low organic matter content which is 3.82%.

The (CCME) has developed soil quality guidelines depending on what the land is to be used for. The agricultural soil quality guidelines are the most restrictive when compared to other guidelines (residential, parkland, commercial or industrial).(https://www.ccme.ca/en/resources/ canadian_ environmental_quality guidelines/index.html).

Heavy metal concentrations within the sampled soils and the CCME quality standards for the agriculture soil are presented in **Table 1.3**.

Table 1.3. Heavy metal contents in soil samples (n=7). Mean values are presented with standard deviation and CCME limits for agricultural soils

Elements	**Concentration (mg kg^{-1})**	**CCME limits for Agriculture Soil (mg kg^{-1})**
Cr	17.18 ± 2.74	64
Ni	20.14 ± 4.26	45
Co	4.59 ± 1.1	40
Cu	8.04 ± 1.8	63
Zn	63.02 ± 7.42	200
As	2.87 ± 0.89	12
Cd	0.25 ± 0.11	1.4
Pb	8.66 ± 1.15	70
Se	ND	1
V	27.47 ± 2.32	130
Hg	ND	6.6

ND = Not detected

In the soil studied, Cd has the lowest mean concentration (0.25 ± 0.11 mg kg^{-1}), while the highest concentrations were recorded for Zn (63.02 ± 7.42 mg kg $^{-1}$). Bioavailability of Zn varies depending on the electrochemical properties of the Zn species and the surrounding environment and is determined by the amount of soluble zinc present (Wuana and Okieimen, 2011). There were no detectable amount of selenium and mercury in the analyzed soil sample. According to the CCME Quality guidelines for agricultural soils, heavy metal concentrations in the studied soil are generally below the threshold values (

Table 1.3) and the soil can be considered for agricultural purpose.

1.5.2 Lime requirement

Liming is the most economical method of ameliorating soil acidity (Schwartz and Follett, 1979). The rate of lime required to bring about a desired pH change is determined by several factors; including (a) the change in the pH required, (b) the buffer capacity of the soil, (c) the chemical composition of the liming material and (d) fineness of the liming material.

Sandy soils and soils low in organic matter have low buffering capacities while clay soils and soils high in organic matter have high buffering capacities. In general, the important soil factors which determine lime requirement include; (i) soil pH, (ii) amount of chemically held acidity in the soil, (iii) depth of cultivation, (iv) desired pH for the crop to be grown, and (v) soil texture (Goulding, 2016)

Results of soil tests in the Atlantic region show that approximately 70% of the soils need from 2 to 8 Mg of lime per hectare to correct present soil conditions. In extreme cases, 16 Mg or even more may be required per hectare (Atlantic Soil Fertility Committee, 1970). Periodic maintenance applications equivalent to approximately 0.5 Mg per hectare per year are required to maintain a given pH (Atlantic Soil Fertility Committee, 1970). Fields that are not uniform in crop growth, texture, color, drainage, or organic matter content are not likely to be uniform in lime requirement.

According to the soil analysis report from the Soil & Plant Laboratory, the following lime application recommendation was made for the studied soil and selected crop species **(Table 1.4).**

Table 1.4 Lime requirements for specific crops

Crop to be grown	Lime requirement ($Mgha^{-1}$)
Legume forage	14.8
Mixed forage	7.3

According to the lime requirement analysis, legume forge need higher lime requirement than mixed forages because legume forages typically require a higher soil pH. For optimum production compared to other forage types, alfalfa, a legume, requires 6.6-7.0 pH, while clovers and birds foot trefoil can withstand slightly more acidic conditions (Turkington and Franko, 1980).

1.5.3 Analysis of fly-ash

1.5.3.1 Fly-ash production in CBPPL

The fuel (biomass, 90% & waste oil, 10%) used in the CBPPL boiler varies depending on boiler conditions and requirements (Churchill and Kirby, 2010). The biomass portion of the fuel mix is a mixture of hog fuel and dewatered secondary sludge from the secondary effluent treatment system. Hog fuel is a mix of coarse chips of bark from trees, sawdust, and wood fibre. Fuel oil is a high-viscosity residual oil used at CBPPL for enhancing the combustibility of wet biomass. Waste oil may contain varying types and amounts of contaminants and used oil amount may affect the contaminant levels in the fly-ash. During the sampling period, on an average about 88% biomass and 12% of waste oil were used as fuel.

1.5.3.2 Characteristics of fly-ash

The physical and chemical properties of fly-ash depend on the various factors (Basu et al., 2009; Demeyer et al., 2001; Kishor et al., 2010; Sahu et al., 2018) such as type of plant, part of plant combusted (bark, wood, leaves), type of waste (wood, pulp or paper residue), the combination with other fuel sources (Demeyer et al., 2001), the condition of combustion (Basu et al., 2009), type of emission control devices and storage and handling (Sahu et al., 2018). Fly-ash is chemically composed of a large number of trace and heavy metals in variable proportions (Basu et al., 2009; Demeyer et al., 2001; Kishor et al., 2010). Analyses of fly-ash have shown the complex and heterogeneous nature of this material. Fly-ash generally has a silt loam texture with 65–90% of the particles having a diameter of less than 0.010 mm (Basu et al., 2009).

Table 1.5 shows the physicochemical characteristic of fly-ash used in this study and the available nutrient percentage.

Table 1.5. Physicochemical characteristics of fly-ash used in this study

Source	pH $CaCl_2$	EC dS m^{-1}	CCE%	Available Nutrients %		
				Mg	Ca	K
CBPPL	10.0	50.3	42	3.01	8.98	4.09

Fly-ashes differ widely in their pH (3.8–12.8) and also their chemical characteristics depend on their sources (Yunusa et al., 2012). pH of the fly-ash is directly related to the availability of macro and micronutrients. Based on the pH, fly-ash has been classified into 3 categories, namely; (1) slightly alkaline 6.5 – 7.5, (2) moderately alkaline 7.5 - 8.5, (3) highly alkaline >8.5. In the present study, pH of fly-ash sample was measured as 10.0; it indicates that fly-ash was highly alkaline in nature and can be used for reclamation of acidic soils.

Determination of the CCE of wood ash is done according the protocol of the Association of Official Analytical Chemists (AOAC) for agricultural limestone. The procedure involves heating a sample in HCl and back-titration of the residual acid with NaOH. Unlike limestone, wood ashes contain not only carbonates but also other components which may react with acid and affect the CCE determination. In general, wood ash has a CCE ranging from 35 to 85% and is commonly used as a liming amendment. In the present study collected fly-ash sample showed CCE 42%.

The concentrations of the macronutrients Ca, K and Mg in the fly-ash from the CBPPL are correspondingly 8, 34 and 11 times higher than in the soil collected from an agricultural field in PBRS. Thus, because of its high nutrient content, the utilization of fly-ash as a nutrient source can also be recommended.

High levels of trace elements (As, Cd, Cr, Pb, Hg, Se, and Mo) seem to present the largest problem for the agricultural use of fly-ash (Aitken and Bell, 1985; Carlson and Adriano, 1993). Regulations in some provinces and states limit the input of heavy metals into soil, and may limit use of fly-ash. The results for heavy metal analysis of fly-ash samples are given in **Table 1.6**.

Table 1.6. The concentrations of the heavy metals in fly-ash from CBPPL. Mean values are presented with standard deviation (n = 12)

Elements	**Concentration (mg kg^{-1})**
Cr	129.84 ± 17.47
Ni	109.90 ± 18.41
Co	19.22 ± 1.63
Cu	197.41 ± 10.76
Zn	1061.63 ± 89.28
As	4.91 ± 0.76
Cd	0.93 ± 0.14
Pb	12.67 ± 0.8
Mo	5.6 ± 1.8
Hg	0.21±0.11
V	58.0 ± 4.5
Se	ND

ND = Not detected, NA = Not available

In the fly-ash sample, Hg has the lowest mean concentration (0.21 ± 0.11 mg kg^{-1}), while the highest content was recorded for Zn (1061.63 ± 89.28 mg kg $^{-1}$). Bioavailability of zinc varies depending on the electrochemical properties of the zinc species and the surrounding environment and is determined by the amount of soluble zinc present. There were no detectable amounts of selenium and mercury observed in the analyzed fly- ash sample.

Due to its physicochemical properties (pH and CCE), this ash can be used as a liming material and would be available to farmers at a very low cost. In order to use this ash as a liming material or soil amendment, it should meet the CCME / CFIA quality guidelines.

1.5.4 Comparison with different guidelines

1.5.4.1 CCME guidelines for compost quality

Two compost categories have been developed for trace element concentrations and foreign matter by CCME (CCME, 2005). These categories (A and B) are based on the end use of the compost material. Category A compost can be used in any application, such as agricultural lands, residential gardens, horticultural operations, the nursery industry, and other businesses. Category B compost has a restricted use because of the presence of higher trace element content. Category B compost may need for additional control when deemed necessary by a province or territory. Compost products that do not meet the conditions for either Category A or B must be used or disposed of properly (CCME, 2005).

Table 1.7. The concentrations of the heavy metals in fly-ash from CBPPL. Mean values are presented with standard deviation (n = 12) and CCME compost guidelines (ND = Not detected)

Elements	**Concentration** (mg kg^{-1})	**CCME compost categories limit (**mg kg^{-1})	
		A	B
Cr	129.84 ± 17.47	210	1060
Ni	**109.90 ± 18.41**	62	180
Co	19.22 ± 1.63	34	150
Cu	197.41 ± 10.76	400	2200
Zn	**1061.63 ± 89.28**	700	1850
As	4.91 ± 0.76	13	75
Cd	0.93 ± 0.14	3	20
Pb	12.67 ± 0.8	150	500
Mo	**5.6 ± 1.8**	5	20
Hg	0.21±0.11	0.8	5
V	58.0 ± 4.5	NA	NA
Se	ND	NA	NA
Tl	ND	NA	NA

Fly-ash analysis results were compared with CCME compost quality guidelines to determine if the fly-ash was suitable as a material for compost. According to one sample t test measured heavy metal concentrations in the fly-ash sample are significantly ($p < 0.000$) below the CCME compost categories B guidelines so could potentially be used in the category B compost. Under the categories B, fly-ash can be used as the soil amendment with additional control.

When compared with the CCME compost categories A guidelines, one sample t test showed that the metals were within the allowable limits (measured heavy metal concentrations in the fly-ash sample were significantly ($p < 0.000$) below the threshold values) except for Ni, Zn and Mo concentrations. The concentration of Ni and Zn in fly-ash were significantly ($p < 0.000$) higher than the compost A guidelines. The mean concentration of Mo was slightly higher than the guidelines values but the difference was not significant.

Once mixed with the other material which are low in above elements (compost, manure, sludge or organic waste), the final compost may pass as a category A compost. However, Zinc maybe present in other natural materials used as an additive, as it is an essential element, present in the tissues of animals and plants even at normal, ambient concentrations. However if plants and animals are exposed to high concentrations of zinc, significant bioaccumulation can results, with possible toxic effects (Wuana & Okieimen, 2011).

1.5.4.2 Canadian Food Inspection Agency (CFIA) fertilizer and supplement metals standards

Accumulation of metals of concern in soil over the long period can have long-term effects on the ecosystem and may cause environmental toxicity. The maximum concentration of metals acceptable in a product depends on the application rate of the product. Metals standards are predicated based on the maximum acceptable cumulative addition to soils over a 45-year time period, as opposed to the actual concentration of the metal in the product (Agriculture and Agrifoods Canada, 1991). The application rate of a product is the main factor in defining acceptable product metal concentrations. The 45-year cumulative application approach is proposed to account for the existence of metals in the environment which eventually determines the level of contamination and thus, long term effects (Canadian Food Inspection Agency, 2017).

Appendix 4 shows the CFIA Fertilizer and Supplement Metals Standards and Examples of Maximum Acceptable Metal Concentrations based on Annual Application Rates. The maximum acceptable product metal concentration (mg metal per kg of product) is calculated by using following equation (Canadian Food Inspection Agency, 2017).

$$A = 10^6 \frac{mg}{kg} \times \frac{maximum\ acceptable\ cumulative\ metal\ addition\ to\ soil\ over\ 45\ years\ (B)}{45\ years\ \times annual\ application\ rate\ (C)}$$

A - Maximum acceptable product metal concentration (mg metal per kg of product)

B - CFIA Standards for maximum acceptable 45-year cumulative metal additions to the soil (kg metal ha^{-1})

C - Product maximum recommended annual application rate (kg product ha^{-1} year^{-1})

Based on the maximum acceptable 45-year cumulative metal additions to the soil (kg metal ha^{-1}), the possible or allowable maximum annual fly-ash application to the field was calculated by using the above equation. Average metal concentration of the fly-ash was assumed as maximum acceptable product metal concentration. **Table 1.8** shows the Maximum Acceptable Cumulative Metal Additions to Soil over 45 Years and Maximum allowable annual application of CBPPL fly-ash based on each element average concentration in fly-ash.

When considering all the elements individually based on their average concentration in the sampled fly-ash, Ni and Zn limit the amount of annual application to the field. According to the result, only 7.27 Mg fly-ash can be applied per hectare field annually. Once mixed with the other components (compost, manure, sludge or organic waste) which are low in above elements (Ni and Zn), the application rate might be increased as smaller the element concentration in fly-ash higher the annual application rate will be.

Table 1.8. CFIA Fertilizer and Supplement Metals Standards and Examples of Maximum Acceptable Metal Concentrations Based on Annual Application Rates ND = Not detected (Canadian Food Inspection Agency, 2017)

Metal	Maximum acceptable cumulative metal additions to soil over 45 years (kg ha^{-1})	Average metal concentration in fly-ash (mg kg^{-1})	Maximum allowable annual application of CBPPL fly-ash Mg $ha^{-1}year^{-1}$
As	15	4.91 ± 0.76	66.67
Cd	4	0.93 ± 0.14	88.89
Cr	210	129.84 ± 17.47	35.90
Co	30	19.22 ± 1.63	33.33
Cu	150	197.41 ± 10.76	16.84
Hg	1	0.21±0.11	111.11
Mo	4	5.6 ± 1.8	15.87
Ni	36	109.90 ± 18.41	7.27
Pb	100	12.67 ± 0.8	170.94
Se	2.8	ND	-
T	1	ND	-
V	130	58.0 ± 4.5	49.81
Zn	370	1061.63 ± 89.28	7.97

1.5.5 Fly-ash application requirement

When ash is used as a liming material, special attention must be given to soil sampling, the lime requirement test and application and incorporation of the ash (Lickacz, 2002). A thorough understanding of each factor is essential to achieve the maximum benefit from the use of fly-ash. With fly-ash, the moisture and purity need to be assessed when determining application rates. According to the fly-ash analysis result and soil lime requirement, fly-ash application rate was calculated by using following Equation 1.

This ash recommendation is based on the lime requirement and specific requirement for the studied soil and selected crop species. This application rate may vary with soil type and its pH, the type of plant to be grown and the quality of ash (CCE % and moisture %). **Table 1.9** shows the lime requirement and the ash recommendation for the studied soil.

Table 1.9. Lime requirement and ash recommendation for the studied soil

Crop to be grown	Lime requirement (Mg ha^{-1})	Ash recommendation (Mg ha^{-1})	Allowable ash application (Mg ha^{-1}) (Based on CFIA standards)	Allowable ash application (%) (Based on CFIA standards)
Legume forage	14.8	36.25	7.27	20
Mixed forage	7.3	17.88	7.27	40.7

According to the CFIA standards, only part of the fly-ash requirement can be substituted by CBPPL fly-ash which is 20% and 40.7% for legume forage and mixed forage, respectively. This percentage can be increased when fly-ash applied with other soil amendments which are low in trace element concentration (Example: Agricultural lime, compost, organic waste and sludge). Some agricultural soils may have low lime requirement or ash requirement depending on their initial pH and the desired pH for the cultivation. The desired pH will be determined by the crop which is going to be grown in the field. Some crops need slightly acidic condition. In those condition most of the lime requirement can be substituted by CBPPL fly-ash.

1.5.6 Present use of wood ash as agricultural soil amendment in Canada

At present, the use of wood ash as a soil amendment is more common on agricultural soils than on forest soils in Canada. Over the last few decades, however, several research trials have been established in British Columbia, Saskatchewan, Manitoba, Ontario and Quebec provinces to examine the effects of wood ash applications on forest soil's physicochemical properties, soil biodiversity, vegetation communities, tree growth and water quality. **Table 1.10** shows the use of wood ash as a soil amendment in Canadian provinces and territories.

Table 1.10. Use of wood ash as a soil amendment in Canadian province and territories (Hannam et al., 2016)

Province	Ash used as a soil amendment?	Common uses of applies ash	
		Purpose	Soil type
Alberta	Yes	Liming	Agriculture
British Columbia	Yes	Liming	Agriculture/Forestry
Manitoba	No	-	-
New Brunswick	Yes	Fertilizing/Liming	Agriculture
Newfoundland & Labrador	No	-	-
Nova Scotia	Yes	-	Agriculture
Northwest Territory	No	-	-
Nunavut	No	-	-
Ontario	No	-	-
Prince Edward Island	No	-	-
Quebec	Yes	Liming	Agriculture/Forestry
Saskatchewan	No	-	-
Yukon Territory	No	-	-

The application of wood ash as a soil amendment in Newfoundland and Labrador (NL) is administrated by the Environmental Protection Act (EPA), including the Environmental Assessment Regulations under the Environmental Protection Act (https://www.assembly.nl.ca/Legislation/sr/Regulations/rc030054.htm). To date, no specific guidance has been developed for wood ash applications on forest or agricultural soils in the province (Hannam et al., 2016). Although soil applications of wood ash are not specifically mentioned in the EPA, it is likely that any large-scale use of wood ash as a soil amendment in NL would require an Environmental Assessment (Hannam et al., 2016).

1.6 Conclusion

Using fly-ash as a soil amendment and liming material can increase soil pH. The metal concentrations values in the CBPPL fly-ash are lower than those in the CCME compost categories B guidelines so that the fly-ash could potentially be used in the category B compost. The concentration of Ni, Zn and Mo in fly-ash were slightly higher than those in the CCME compost A guidelines. If the fly-ash can be mixed with the other soil amendments which are low in these elements prior to land application, then the final product may pass as a category A compost. According to the CFIA standards, only part of the ash requirement can be substituted by CBPPL fly-ash. The percentage may vary depending on soil initial soil pH and desired pH and which crop is to be grown. This percentage can be increased when fly-ash applied with other soil amendments which are low in trace element concentrations. Also, maximum allowable ash can be applied annually to forest land and marginal land to improve their fertility level and productivity.

1.7 Recommendations

Continuous fly-ash sampling and analysis will be required throughout the year to monitor the temporal variability of fly-ash quality. Specific guidelines should be developed for fly-ash applications on forest and/or agricultural soils in the province of NL. However, the estimation of the leaching potential and the bioavailability of toxic elements is important in assessing the possible environmental impact associated with the utilization of fly-ash.

1.8 References

Acton, D.F., Gregorich, L.J., (1995). The Health of Our Soils: Toward Sustainable Agriculture in Canada. Agriculture Agri-Food Canada, CDR Unit, Ottawa.

Ahmad, A. and Alam, M.M. (1997). Utilization of fly-ash and Paecilomyces lilacinus for the control of Meloidogyne incognita. International Journal of Nematolology 7: 162–164

Aitken RL, Bell LC. (1985) Plant uptake and phytotoxicity of boron in Australian fly ashes. Plant Soil 84:245–257

Alberta Environment. (2002) Standards and Guidelines for the use of wood ash as a liming material for agricultural soils

Atlantic Soil Fertility Committee. (1970). Atlantic soils need lime. N.B.: Published by authority of the Deputy Ministers of Agriculture of N.B., N.S., P.E.I. and Nfld.

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y., & Dhiba, D. (2018). Soil Microbial Resources for Improving Fertilizers Efficiency in an Integrated Plant Nutrient Management System. 9(July). https://doi.org/10.3389/fmicb.2018.01606

Basu A., Saha D., Saha R., Ghosh T., Saha B. (2014). A review on sources, toxicity and remediation technologies for removing arsenic from drinking water. Res. Chem. Inter med. 2014;40:447–485.

Brown, Ray W.; Amacher, Michael C.; Mueggler, Walter F.; Kotuby-Amacher, Janice. (2003). Reestablishing natural succession on acidic mine spoils at high elevations: long-term ecological restoration. Res. Pap. RMRS-RP-41. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 49 p

Campbell AG. (1990) Recycling and disposing of wood ash, Tappi J, vol. 73 9(pg. 141-146)

Carlson, C.L. and Adriano, D.C. (1993). Environmental impacts of coal combustion residues. Journal of Environmental Quality 22: 227–247.

Chang AC, Lund LJ, Pagek AL, Warneke JE. (1977). Physical properties of fly ash amended soils.

Churchill, D. and Kirby, A. (2010) CB Pulp and Paper Ash Diversion, Phase 1: Initial Ash Testing, Project No 09-14.

Clapham, W.M., and L.M. Zibilske. (1992). Wood ash as a liming agent. for plant analysis. CRC Press, New York. Commun. Soil Sci. Plant Anal. 23:1209–1227

Demeyer, A., Voundi Nkana, J.C., Verloo, M.G. (2001). Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour Technol. 77, 287–295

Department of Environment and Climate Change. (2017). Government of Newfoundland and Labrador. http://www.ecc.gov.nl.ca/env_protection/ics/pulp.html

Goulding, K.W.T. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manag. 32, 390–399.

Hackett, G. A. R., Easton, C. A., & Duff, S. J. B. (1999). Composting of pulp and paper mill fly ash with wastewater treatment sludge. Bioresource Technology, 70(3), 217–224. https://doi.org/10.1016/S0960-8524(99)00048-6

Hao, X., Ball, B.C., Culley, J.L.B., Carter, M.R., Parkin, G.W. (2007). Soil Density and Porosity, in: Gregorich, E.G., Carter, M.R. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, pp. 743–759.

Hendershot, W.H., Lalande, H., Duquette, M. (2007). Soil Reaction and Exchangeable Acidity, in: Gregorich, E.G., Carter, M.R. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, pp. 173–178.

Huang, H., Campbell, A.G., Folk, R., Mahler, R.L. (1992). Wood ash as a soil additive and liming agent for wheat. Field studies. Commun. Soil Sci. Plant Anal. 23, 25±33

Jala S, Goyal D (2006) Fly-ash as a soil ameliorant for improving crop production – a review. Bioresour Technol 97:1136–47

Jukić, M., Ćurković, L., Šabarić, J.n(2017)nEnviron Contam Toxicol 99: 524. https://doi.org/10.1007/s00128-017-2160-0

Kahl, J.S., Fernandez, I.J., Rustad, L.E., Peckenham, J., 1996. Threshold application rates of wood ash to an acidic forest soil. J. Environ. Qual. 25, 220-227

Karak, T., Das, D. K., Singh, U. K., and Maiti, D. (2005). Influence of pH on soil charge characteristics and cadmium sorption in some noncontaminated soils of Indian subtropics. The Scientific World Journal 5, 183–194

Karpagavalli, S. and Ramabadran, R. (1997). Effect of lignite fly ash on the growth and dry matter production (DMP) of soil borne pathogens. In: Souvenir and Abstract: National Seminar on Bio-Utilization of Fly Ash. Khallikote Autonomous College, Berhampur, Orissa, India, April 4–5, p. 11

Khan, M.R. and Singh, W.N. (2001). Effects of soil application of fly-ash on the fusarial wilt of tomato cultivars International Journal of Pest Management 47 (4): 293–297.

Khan, N.R.; Khan, M.W. and Singh, K. (1997). Management of fly ash in soil. Plant Pathology 46: 33–43.

Kishor, P., K, G. A., & Dileep Kumar. (2010). Use of Fly-ash in Agriculture: A Way to Improve Soil Fertility and its Productivity. Asian Journal of Agricultural Research, 4, 1–14.

Krejsl, J.A., and T.M. Scanlon. (1996). Evaluation of beneficial use of wood fired boiler ash on oat and bean growth. J. Environ. Qual. 25:950–954

Kroetsch, D., Wang, C. (2007). Particle Size Distribution, in: Gregorich, E.G., Carter, 72 M.R. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, pp. 713–726.

Lerner, B.R., Utzinger, J.D. (1986). Wood ash as soil liming material. Hort Science 21, 76-78.

Lickacz, J. (2002). Wood Ash - An Alternative Liming Material for Agricultural Soils. (February), 1–6.

Lohry, R. (2007): Micronutrients: functions, sources and application methods. Indiana CCA Conf. Proceeding.

Lombin LG (1979) Evaluation of the magnesium supplying powers of the Nigerian savanna soils. Soil Sci Plant Nutr 25:477– 492

Meiwes, K.J. (1995). Application of lime and wood ash to decrease acidification of forest soils. Water Air Soil Pollut. 85, 143-152

Miller, J.J., Curtin, D. (2007). Electrical Conductivity and Soluble Ions, in: Gregorich, E.G., Carter, M.R. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, pp. 161–172

Muse, J.K., Mitchell, C.C. (1995). Paper mill boiler-ash and lime byproducts as soil liming materials. Agron. J. 87, 432-438

Naylor, L.M., Schmidt, E.J. (1989). Paper mill wood ash as a fertilizer and liming material: Field trials. Tappi J. 72, 199-206

Ohno, T., Erich, M.S. (1990). Effect of wood ash application on soil pH and soil test nutrient levels. Agric. Ecosyst. Environ. 32,223-239.

Patterson, S. (2001). The agronomic benefits of pulp mill boiler wood ash. MSc thesis, University of Lethbridge, Lethbridge, AB. 39 pp.

Pichtel, J.R. (1990). Microbial respiration in fly ash/sewage sludge-amended soils. Environ. Pollut., 63, 225-237.

Pitman, R. M. (2006). Wood ash use in forestry - A review of the environmental impacts. Forestry, 79(5), 563–588. https://doi.org/10.1093/forestry/cpl041

Pöykiö R, Kuokkanen T & Nurmesniemi H (2004) Fly ash from Pulp and Paper Mills: A Potential Soil Amendment and a Forest Fertilizer. In: Pongrácz E. (ed.): Proceedings of the Waste Minimization and Resources Use Optimization Conference. June 10th 2004, University of Oulu, Finland. Oulu University Press: Oulu. p. 171-177.

Rautaray SK, Ghosh BC, Mittra BN (2003) Effect of fly ash, organic wastes and chemical fertil-izers on yield, nutrient uptake, heavy metal content and residual fertility in a rice-mustard cropping sequence under acid lateritic soils. Bioresource tchnol 90:275-283

Sahu, G., Chandra, B., Viswavidyalaya, K., Bag, A. G., Chandra, B., Viswavidyalaya, K., … Viswavidyalaya, K. (2018). Potential use of flyash in agriculture : A way to improve soil health Potential use of flyash in agriculture : A way to improve soil health. Journal of Pharmacognosy and Phytochemistry, 6(September), 873–880

Sarangi, P.K.; Mishra, T.K. and Mishra, P.C. (1997). Soil metabolism fly-ash amended soil. Indian Journal of Environmental Science 1(1): 17–24

Schwartz, J. W., and R. F. Follett. (1979). Liming acid soils. Washington: Dept. of Agriculture, Office of Governmental and Public Affairs

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. Indian Journal of Pharmacology, 43(3), 246–253.

Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences*, *22*(2), 123–131. doi:10.1016/j.sjbs.2014.12.001

Topp, G.C., Parkin, G.W., Ferre, T.P.A. (2007). Soil Water Content, in: Gregorich, E.G., Carter, M.R. (Eds.), Soil Sampling and Methods of Analysis. CRC Press, pp. 939–961.

Turkington, R. & Franko, G. D. (1980). The biology of Canadian weeds. 41. Lotus corniculatus L. Can. J. Plant Sci. 60: 965–979.

Ulery, A.L., Graham, R.C., Amrhein, C. (1993). Wood-ash composition and soil pH following intense burning. Soil Sci. 156, 358- 364.

Unger, Y.L., Fernandez, I.J. (1990). The short-term effects of wood-ash amendment on forest soils. Water Air Soil Pollut. 49, 299-314

Williams, T.M., Hollis, C.A., Smith, B.R. (1996). Forest soil and water chemistry following bark boiler bottom ash application. J. Environ. Qual. 25, 955-961

Wong JWC, Wong MH (1990) Effects of fly-ash on yield and elemental composition of two vegetables, Brassica parachinensis and B.chinensis. Agric Ecosys Environ 30:251–264

Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. https://doi.org/10.5402/2011/402647

Yunusa, I. A. M., Loganathan, P., Nissanka, S. P., Manoharan, V., Burchett, M. D., Skilbeck, C. G., & Eamus, D. (2012). Application of coal fly ash in agriculture: A strategic perspective. Critical Reviews in Environmental Science and Technology, 42(6), 559–600. https://doi.org/10.1080/10643389.2010.520236

Chapter 2: Effect of Biochar on Leaching and Bioavailability of Heavy Metal on Fly-ash Amended Soil

Introduction

2.1 Background

Fly-ash is a by-product generated by pulp and paper companies, lumber manufacturing plants and utilities that burn wood products, bark and paper mill sludge as a means of disposal and/or energy production. Large quantities of fly-ash are generated by these industries since combustion of wood generally produces 6 to 10% ash (Pitman, 2006). Most of these ashes are landfilled or discarded in lagoons. However, the increasing expense of landfill disposal has led to increased interest in the land application of industry generated fly-ash. Corner Brook Pulp and Paper Limited (CBPPL) mill is producing around 15,000 Mg of fly-ash currently in each year (Churchill & Kirby, 2010). Ash produced is being disposed of at a local landfill site.

There is a high potential for fly-ash to be used as a soil amendment in agricultural lands (Demeyer et al., 2001) due to its high pH (Campbell, 1990; Pitman, 2006), the potential to raise the pH of acidic soils (Naylor & Schmidt, 1989; Williams et al., 1996), nutrient availability (Camberato et all., 2011; Demeyer et al., 2001; Kishor & Kumar, 2010) and increasing cost of lime and chemical fertilizers (Sharifi et al., 2013). There are various benefits to diverting the ash generated by the pulp and paper industry as an alternative liming agent or soil amendment (Lickacz, 2002). They would save on disposal fees. There would be environmental benefits because of reduction of material landfilled, saving of groundwater from potential contamination and it will reduce the liming cost for the farmers due to its easy access and low cost.

In addition, past studies have shown that fly-ash from pulp and paper mill would have some metal contaminants (Pöykiö et al., 2005; Pitman, 2006) which can have long-term effects on the ecosystem (Singh et al., 2011) when we apply to agriculture soil as a soil amendment. Heavy metals are not biodegradable and persist for a long time in contaminated soils and it is expensive and time-consuming to remove heavy metals from contaminated soils (Singh et al., 2011).

That may limit the use of fly-ash as a soil amendment. These inorganic contaminants might spread to surrounding environments through leaching to groundwater or even surface water (Puga et al., 2016). In order to diminish the harmful effects of heavy metals, it is essential to control the soluble and exchangeable fractions of metals in soils. Stabilization of heavy metals *in situ* by adding soil amendments such as lime and compost is commonly employed to reduce the bioavailability of metals and minimize plant uptake (Bolan and Duraisamy, 2003; Kumpiene 2010)

Biochar can improve the quality of the contaminated soil by reducing the mobility and bioavailability of heavy metals in the contaminated soils (Ippolito et al., 2012) and thereby could cause a significant reduction in crop uptake of heavy metals. Application of biochar can potentially provide a new solution for control of the soils contaminated by heavy metals. Biochar is a product of biomass pyrolysis; heated under minimal (Abujabhah et al., 2016) or no oxygen supply (Houben et al., 2013; Lehmann and Joseph, 2009). Some recent studies have shown promising results related to the *in-situ* stabilization of heavy metals in contaminated soils with the application of biochar (Beesley et al., 2010; Uchimiya et al., 2010; Fellet et al., 2011; Karami et al., 2011; Park et al., 2011). Because of biochar's highly porous structure, active functional groups (Zhou et al., 2016; Zhang et al., 2017) and generally high pH and cation exchange capacity (Zhang et al., 2013; Zhang et al., 2017; Park et al., 2011) biochar shows a great affinity for heavy metals (Mohanet al., 2007; Cao et al., 2009; Park et al., 2011). The principal mechanisms of metal immobilization by biochar in soils include an increase in soil pH, ion exchange, physical sorption and precipitation (Beesley et al., 2011) as oxi-hydroxides, with carbonate or phosphate(Puga et al., 2016; Park et al., 2011; Uchimiya et al., 2010).

Furthermore some recent studies have shown that the application of biochar in soils rapidly increases the soil fertility (Chen et al., 2011) and plant growth (Ibrahim et al., 2013) by supplying and retaining nutrients (Beesley et al., 2011) while improving soil physiochemical and biological properties (Beesley et al., 2011; Abujabhah et al., 2016; Glaser et al., 2002; Novak et al., 2009).

Therefore, biochar has been considered as a potential amendment for our study. This study aims to assess the potential of fly-ash as alternate liming material while developing an eco-friendly approach to reduce or eliminate the heavy metal leaching and plant uptake from fly-ash amended soil with incorporation of biochar.

2.2 Biochar as a soil amendment

2.2.1 Biochar properties

According to past studies, conducted over the last few years, biochar has shown good potential as a soil amendment favouring heavy metal immobilization (Bashir et al., 2017; Houben et al., 2013; Lu et al., 2014; Wang et al., 2017; Melo et al., 2013; Cao et al., 2009; Li et al., 2009). Biochar is a product made after thermal decomposition of organic material under limited supply of oxygen and a temperature below 900°C (Lehmann and Joseph, 2009). Biochar can be obtained from pyrolysis of plant-derived biomass (wood bark, rice husk, pinewood etc.) or non-plant derived biomass (dairy and chicken manure) (Godlewska et al., 2017). Also, it can be produced in gasifiers, be co-product or by-product in retorts and biogas and bio-oil technologies (McLaughlin et al., 2009).

Biochar is produced under various pyrolysis production conditions. Pyrolysis conditions such as heating rate, highest treatment temperature, pressure, reaction residence time, reaction vessel, pre-treatment, post-treatment can be varied and will affect the properties of the biochar produced. Temperature and feedstock are considered the most important factors affecting biochar properties (Downie et al., 2009).

Biochar surface area has numerous micropores with < 2 nm diameter, which gives adsorptive properties to biochar (Downie et al, 2009). The surface area and pore size grow with the temperature because of functional groups destruction (Angin and Sensöz, 2014). However, at a certain temperature point, some deformation occurs, and surface area decreases (Downie et al., 2009). Uchimiya et al. (2011) and Chun et al. (2004) detected these changes at temperature above 700°C. Pore size can influence metal sorption as metals cannot be adsorbed by very small pores (Ahmedna et al., 2004).

Elemental composition of biochar depends on pyrolysis temperature. Generally, biochar has a high C content with a high amount of aromatic structures (Lehmann and Joseph, 2009). At higher temperatures, C content normally increases, and structure becomes more condensed (Angin and Sensöz, 2014). Moreover, process of C graphitization, dehydration, and deoxygenation of biomass take place (Ahmad et al., 2014; Mendonça et al., 2017). Oxygen content decreases with the temperature because of the decomposition of oxygen surface groups (Mendonça et al., 2017). The amount of volatile matter content also decreases with high temperature (Hagner et al., 2016).

pH of the biochar depends on pyrolysis temperature and feedstock properties (Li et al., 2017). Generally, pH of biochar has alkaline values and rises with the pyrolysis temperature (Angin and Sensöz, 2014) because of the formation of ash. Moreover, the number of base cations is higher at higher temperature, favouring pH increase (Yuan et al., 2011). High temperatures favour depolymerization of biomass (Keiluweit et al., 2010), but this process is not observed in the biochars from non-plant feedstocks because they do not have lignocellulosic molecules (Ahmad et al., 2014). After being added to the soil, biochar interacts and aggregates with mineral and organic matter. Possible degradation may occur due to biotic degradation of a labile biochar fraction, erosion, leaching, and pedoturbation (Lehmann et al., 2009). However, biochar is very stable in the environment because of its organo-chemical and physical structure. For example, biochar produced during forest fires could be more than 10,000 years old (Lehmann et al., 2009). Kuzyakov et al. (2009) suggested that biochar residence time in soils of temperate climates is about 2,000 years.

2.2.2 Environmental benefits of biochar application to the soils

Biochar application can contribute to solving various environmental problems – greenhouse gases emissions, high CO_2 concentration in the atmosphere, and managing organic waste (Godlewska et al., 2017; Ahmad et al., 2014). Biochar is also applied for soil improvement and energy production (Lehmann and Joseph, 2009; Ahmad et al., 2014; Sohi et al., 2012). Biochar is considered as environmentally friendly ameliorant as local and renewable resources are used for its production (Lehmann and Joseph, 2009). Various waste biomass including crop residue, manure and sludge are used for biochar production (Xu et al., 2013; Cao et al., 2009).

The usage of biochar favours managing this waste and reduces pollution loading to the environment. During the process of charring the volume and mass of the waste is reduced. Another benefit is decreasing emissions of the greenhouse gas methane from the landfill, if the biomass had simply been left to decompose (Lehmann and Joseph, 2009; Ahmad et al., 2014). Moreover, pyrolysis processes reduce pathogenic microorganisms from sludge or manure biochar (Lehmann and Joseph, 2009; Ahmad et al., 2014). It also has the benefit of decreasing energy used in the long-distance transport of waste (Lehmann and Joseph, 2009; Ahmad et al., 2014). Biochar application to agricultural lands can sequester atmospheric carbon dioxide and mitigate climate change (Lehmann and Joseph, 2009; Godlewska et al., 2017).

Biochar is very stable in soils due to its physical structure (Kuzyakov, 2009; Lehmann and Joseph, 2009). This long-term stability plays an important role in reducing CO_2 emissions as it decreases the rate at which photosynthetically fixed carbon is transmitted to the atmosphere (Woolf et al., 2010). Biochar was suggested as a sink for atmospheric CO_2 by Glaser et al. (2002). Zweiten et al. (2009) also reported a reduction of CH_4 emissions by biochar application (Zweiten et al., 2009). Globally, biochar implementation can reduce total greenhouse emissions by 12% annually (Woolf et al., 2010).

2.2.3 Effect of biochar on soil properties and plant growth

Biochar can positively affect soils physical and chemical properties (ion exchange capacity, porosity, water holding capacity, retention of nutrients or microbial activity) (Godlewska et al., 2017; Hussian et al., 2016; Glaser et al., 2002). According to Glaser et al. (2002) and Verheijen et al. (2010), biochar addition to soil increases its cation exchange capacity and improves higher nutrient retention in comparison to untreated soil.

Moreover, biochar has a low bulk density, therefore its addition can reduce the bulk density of soil and improve soil structure. Also, biogeochemical processes in soils are altered after biochar addition due to changes in microbial communities and changes in enzyme activities (Ahmad et al., 2014). All these processes improve soil fertility and provide better crop productivity after biochar addition (Glaser et al., 2002). Water retention of soil increases after biochar application (Downie et al., 2009) because net soil surface area increases (Verheijen et al., 2010). Glaser et al., (2002) suggested increase in water holding capacity after biochar additions because of high organic matter content in biochar (Glaser et al., 2002). This effect is long-term because of biochar's stability and recalcitrance (Verheijen et al., 2010). Due to additional water and nutrients in the micropores, biochar may improve plant water availability, especially for sandy soils during dry periods. However, in the case of small pores, biochar particles can block soil pores, reducing the infiltration ability of soil (Verheijen et al., 2010).

Soils organic matter is significantly altered by the addition of biochar (Ahmad et al., 2014). For instance, it can cause a positive priming effect (Zimmerman et al., 2011) by faster decomposition of soil native C by changing microbiological conditions (Kuzyakov at al., 2009). In other cases, the negative priming effect was observed due to the adsorption of dissolved organic C and its slower decomposition.

Studies have demonstrated that biochar is important for improving the beneficial microbial populations in the soil. Because of the highly porous structure and large surface area of biochar, it can harbor beneficial soil micro-organisms such as mycorrhizae and bacteria and enhance the binding sites for nutrients (Atkinson et al., 2010). Therefore, it would increase the bioavailability and plant uptake of key nutrients.

Major et al. (2010) studied the effect of single application of biochar on Colombian savanna Oxisol for four years, and found that maize yield did not significantly increase in the first year but increased by 28, 30 and 140% for 2004, 2005 and 2006 respectively at the rate of 20 Mg ha^{-1} in comparison to the control. Furthermore, they reported that higher crop yield and nutrient uptake was primarily due to the 77–320% increase of available Ca and Mg in the soil where biochar was applied.

2.2.4 Biochar application for heavy metal immobilization

Biochar is considered as a soil amendment having a great potential for immobilizing heavy metals (Ahmad et al., 2014; Xu et al., 2012, Al-Wabel et al., 2015). However, the ability of biochar to adsorb contaminants varies depending on biochar's physico-chemical properties and target pollutant (Ahmad et al., 2014). The most important parameters are feedstock and pyrolysis temperature. Biochar can be produced from a great variety of feedstock (e.g. wood, grain residues, straw, nutshells, seeds/pips, poultry and cattle manure, paper pulp and sewage sludge), using locally available and renewable materials can be considered a truly viable option for rural areas in developing countries. Several mechanisms have been proposed to govern metal sorption by biochar from contaminated soil or soil solution **(Figure 2.1**).

The main mechanisms of reducing metal mobility include processes of complexation with functional groups, cation exchange with biochar surface, precipitation and formation of insoluble species, electrostatic attraction to biochar surface, reduction and further sorption of reduced compounds (Li et al., 2017; Ahmad et al., 2014). Also, often, these mechanisms can act at the same time. Ahmad et al. (2014) suggested that low temperature pyrolysed biochar with the high amount of O-containing functional groups generally show good efficiency for heavy metal stabilization.

The mineral components such as phosphates and carbonates in biochar play an important role in stabilization of heavy metals in soils because these salts can precipitate with heavy metals and reduce their bioavailability (Cao et al., 2009). For example, precipitation is the main mechanism of cadmium (Cd) immobilization by biochar (Xu et al., 2013). Biochars derived from various feedstocks can have different mechanisms of metal sorption. For instance, manure biochar can contain high amounts of carbonate and phosphate.

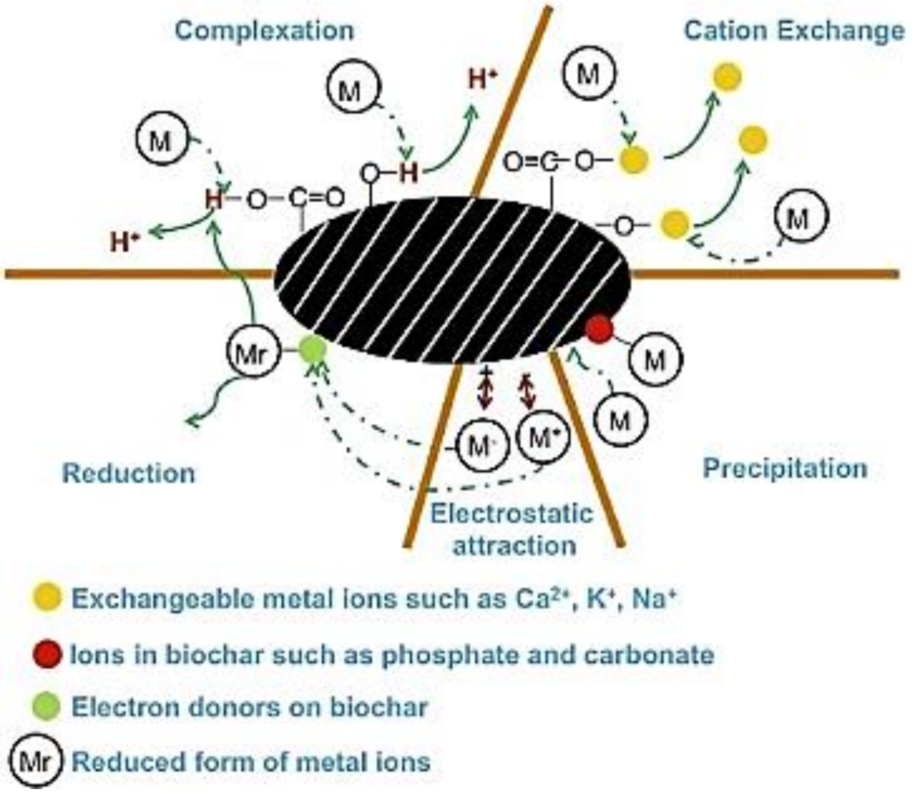


Figure 2.1: Conceptual illustration of heavy metal sorption mechanisms on biochar (Li et al., 2017)

Cation exchange can predominate in cases of biochar having relatively high CEC and high amount of calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) (Harvey et al., 2011; Li et al., 2017). In the case of biochars with low CEC, other mechanisms play an important role in heavy metal immobilization. For example, in Cd sorption complexation with carboxylic surface functional group and precipitation are important. Uchimiya et al. (2011) observed the important role of carboxyl, hydroxyl, and phenolic functional groups for metal binding. Moreover, CEC in soil increases after adding biochar amendment and soil pH range shifts towards neutral and alkaline range. Under these conditions metal mobility decreases, and the mobilization of oxyanions increases (Al-Wabel et al., 2015; Lu et al., 2014).

There are still some uncertainties in biochar application for heavy metal immobilization. Many studies focused on soils, which were spiked with heavy metals under laboratory conditions. There is still a lack of research on aged field contaminated soils (Lu et al., 2014). Moreover, biochars vary greatly in their properties and ability to adsorb the contaminants. More field studies and *in-situ* experiments are required for interpreting the mechanisms of various biochars and long-term effect on the contaminated soils.

2.3 Objective

2.3.1 General objective

The general objective of this study was to assess the potential of biochar to reduce or eliminate the heavy metal mobility/leaching from the fly-ash amended soils.

2.3.2 Specific objective

The specific objectives of this study were

- To evaluate the impact of biochar incorporation into fly-ash amended soil on heavy metal leaching using soil columns
- To assess the effect of biochar incorporation into fly-ash amended soil on heavy metal bioavailability and plant growth

2.4 Materials and Methodology

2.4.1 Biochar sample collection and characterization

The required amount of biochar was obtained from AirTerra Inc. pH, moisture content, ash, CEC, of the biochar were analyzed by AirTerra Inc. See **Table 2.1** for biochar characteristics.

2.4.2 Soil preparation

The required soil for this study was sampled from a land which is under natural vegetation in the Pynn's Brook Research Station (PBRS), Pasadena, NL, Canada. The site is located (49°04'23"N, 57°33'39"W) in the Humber Valley Watershed in the western part of the island of Newfoundland. The soil samples were collected from shallow depths (0-30 cm). The collected soil sample was air-dried for one week and sieved to a particle size of <2mm in diameter. Based on the soil’s lime requirement and fly-ash analysis (CCE, moisture content) results from the previous chapter, fly-ash was thoroughly mixed with soil manually at the rate of 15 g fly-ash per 1000 g soil which is equivalent to 36.25 Mg ha^{-1} (Ash requirement for legume forage **Table 1.9**).

2.4.3 Leaching column experiments

A leaching column experiment was conducted in order to assess the mobility and leachability of heavy metal in biochar amended soil at the Soil Science laboratory, Grenfell Campus, Memorial University of Newfoundland. The experiment was carried out at room temperature, under laboratory conditions.

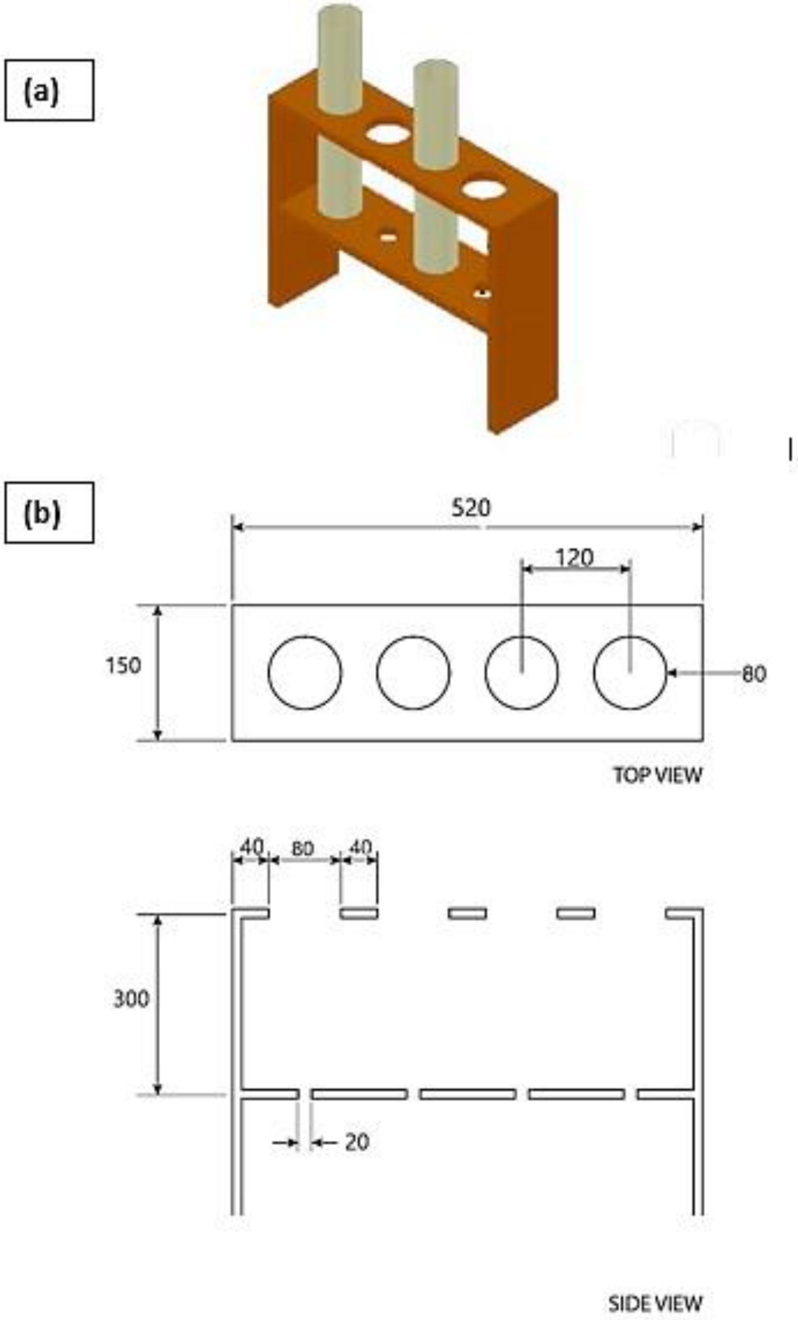


Figure 2.2: Detailed drawing of experimental setup showing (a) 3D view (b) the top and side view of the experiment setup (all dimensions are in mm**)**

2.4.4 Experimental Design

A treatment structure with four rates of biochar application was used. The fly-ash amended soil was manually mixed with 0, 2.5, 5, and 10% (w/w) biochar. The above treatments were arranged in a completely randomized design, with three replicates for each treatment for a total of 12 experimental units (**Figure 2.2**) in a custom-built set-up**.** The leaching columns (D = 8 cm, H = 40 cm) were filled with each of the treatments described above for a height of 30 cm which allowed for a dry bulk density of 1.3 g cm^{-3}. The soil column was filled by placing 10 equal layers to ensure uniform density. After placing each loose soil layer, a plastic plate was placed over the soil and a mass of x kg was dropped onto the plate from a height of 20 cm above the soil layer to pack the material in the column to a 30 cm height. After the column was packed with soil, the column was saturated with water from bottom to top to avoid trapping air bubbles. Then 210.0 mL of water (0.5 pore volume of soil) was applied in each leaching event for a total of 11 consecutive leaching events (**Figure 2.3**). Each column was flushed with a total of 2310 mL (equivalent 734.5 mm of rainfall) of water, which corresponds to one year’s average precipitation (rain) in the study area calculated over 25 years (https://climate.weather.gc.ca).

Figure 2.3: The front view of the experimental setup showing the leaching columns and the leachate collecting system

2.4.5 Assessing the heavy metals leaching during leaching events

Leachates were collected from each leaching event separately using 250mL plastic bottles **(Figure 2.4)** and the volume for each was measured. To prepare the collected leachate for analysis it was filtered through a 0.45 mm membrane filter and acidified by adding concentrated HNO_3 to bring the sample pH below 2. Leachate samples collected from column leaching tests were analysed for copper (Cu), lead (Pb), zinc (Zn), Molybdenum (Mo), iron (Fe), chromium (Cr), nickel (Ni), mercury (Hg), vanadium (V), Arsenic (As), cobalt (Co) and Cd by ICP-MS. The detection limit for all heavy metals were 0.05 µg L^{-1}. All the reagents used for analysis were of analytical grade or higher. All the containers were soaked in 10% HCl, rinsed thoroughly in deionized water, and dried before use. The standard substances such as the multi element standard solution was used to examine the precision and accuracy of determination.

Figure 2.4: Collected leachate during one of the leaching events

The total heavy metal mass leached from each column during the leaching event was calculated by using the leached volume from each leaching event and the concentration of metal in each leachate. Before and after the leaching process, the total heavy metal contents in each soil treatment was analyzed by using the ICP-MS (EPA method 3050B). For determination of total heavy metal content of the soil samples, 1 g of each soil sample (oven dried at 70˚C for 48 h) was accurately weighed into a digestion tube and 10 mL concentrated nitric acid: water, 1:1 v/v added. The sample was then heated at 95˚C on a heating block (2006 Digestor, Foss Tecator) for15 min without boiling. After cooling at room temperature for 5 min, 5 mL concentrated HNO_3 was added and the sample was heated at 95^{o}C for 30 min. Additional 5 mL aliquots of concentrated HNO_3 was added until no brown fumes were given off. The solution was then allowed to evaporate to <5 ml. After cooling, 2 mL of water and 3 mL of 30% H_2O_2 were added and heated until effervescence subsided, and the solution cooled. Additional H_2O_2 was added until effervescence ceased (but no more than 10 ml H_2O_2 was added). This stage was continued for 2 h at a temperature less than boiling point. Then, the solution was allowed to evaporate to <5 ml. After cooling, the sample was filtered through Whatman No. 41 filter paper into a 50 ml volumetric flask, and then made up to the mark with distilled water.

2.4.6 Assessing the downward movement of the heavy metal throughout the soil column

In order assess the heavy metal movement through the soil column, after completion of all leaching events, soil from each column was removed and divided lengthwise into three equal sections without mixing or disturbing. As shown in the **Figure 2.5.**

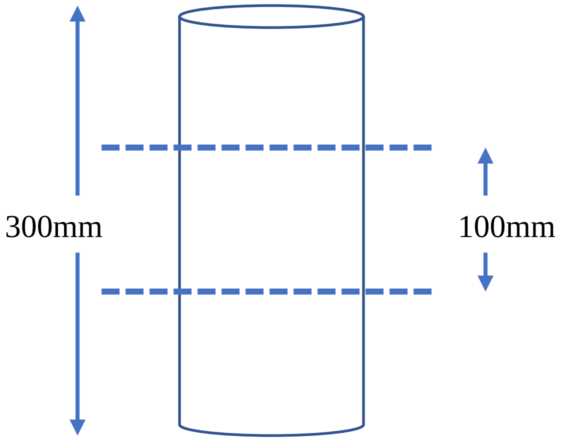


Figure 2.5: Diagram showing the separation of soil column into three equal section

Then each section was thoroughly mixed by hand and air-dried for one week. The air-dried soil was analyzed for heavy metal concentration (V, Cr, Ni, Cu, Zn, As, Cd, Co and Pb) by undergoing acid digestion followed by analysis using ICP-MS using the same method (EPA method 3050B) as before.

2.5 Pot experiments

In order to assess the mobility and the bioavailability of heavy metals in biochar amended soil with fly-ash, pot experiments were conducted under laboratory conditions from January 2018 to April 2018 at the soil science laboratory, Grenfell campus, Memorial University of Newfoundland.

2.5.1 Experimental Design

A treatment structure was used with two plants, Timothy (*Phleum pratense* L.) and Red Clover (*Trifolium pratense*) which are commonly cultivated by Newfoundland farmers, and four rates of biochar application (0, 2.5, 5, 10% (w/w)) with fly-ash amended soil (see section 3.2). The treatments were arranged in a completely randomized factorial design with three replications for a total of 24 experimental units. Prepared fly-ash amended soil were thoroughly mixed manually with different biochar percentages and each plastic pot (Dia =15 cm, V = 2000 cm^3) was filled with the required soil mixture **(Figure 2.6**).

Figure 2.6: Pot experiment showing each pot filled with fly-ash amended soil with different % of biochar

The Timothy and Red Clover seeds used were obtained from the Halifax Seed Company Inc. Twenty seeds were seeded directly into each pot. Ten days after the germination, the seedlings were thinned to ten plants per pot **(Figure 2.7)**. The pots were irrigated with same amount of water (250 mL) in every second day.

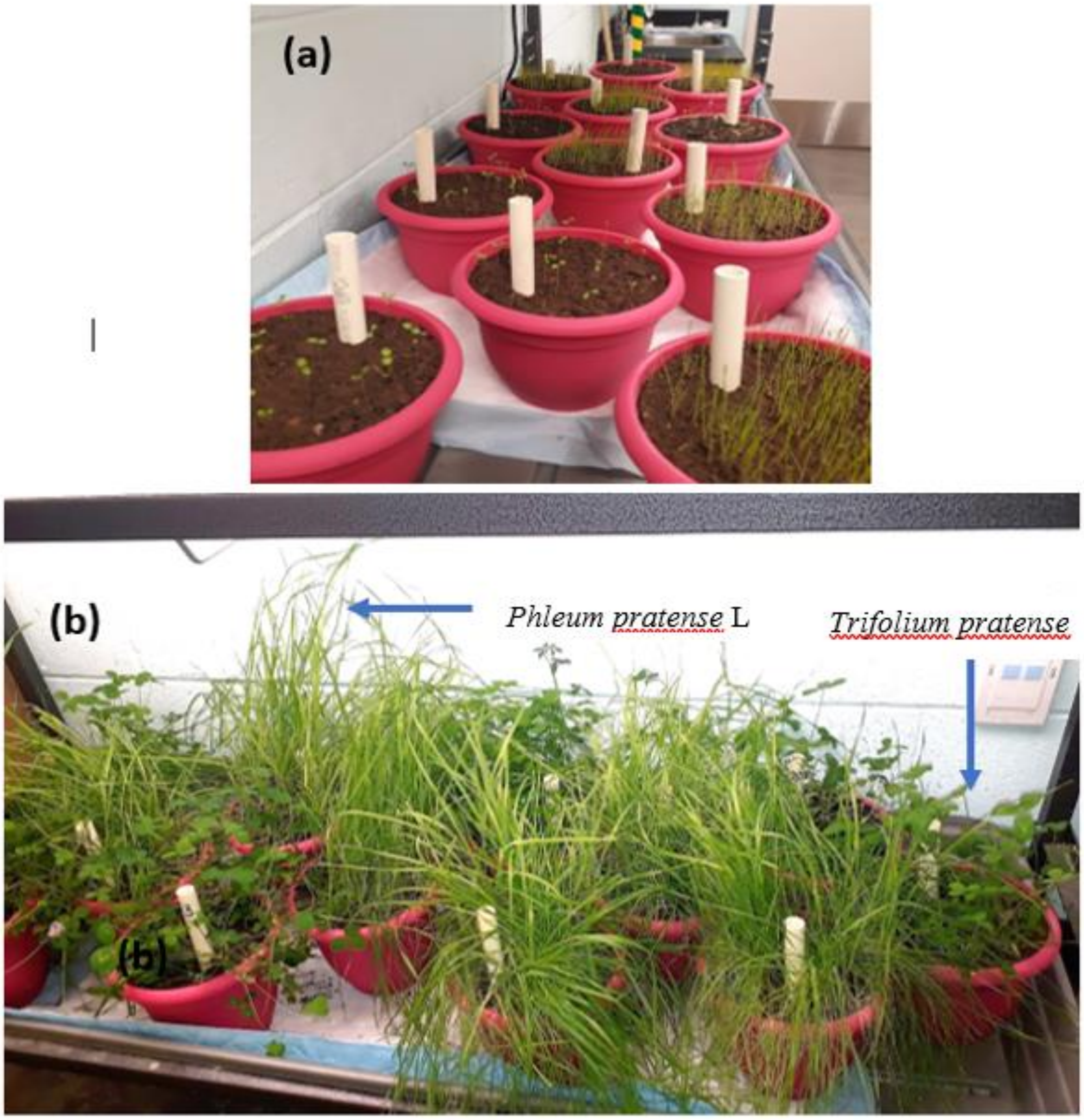


Figure 2.7: Pot experimental unit showing (a) after 10 days of seeding (b) after 90 days of seeding

At the end of the experiment, the above ground portion and roots of the plants were harvested, separated, thoroughly washed by deionized water and dried for one week. Dry ashing was performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. Then the heavy metal contents (Cu, Co, Ni, Cr, V, As, Fe, Mo, Zn, Cd, Hg and Pb) were analyzed by using ICP-MS (EPA method 3050B) after acid digestion using the method described in section 3.3.2.

2.6 Statistical analysis

Statistical analyses to compare the average results of different treatments were performed using a one-way analysis of variance (ANOVA). The Statistical analyses were carried out by using SPSS/Minitab version 2010.

2.7 Results and discussion

2.7.1 Characteristics of Biochar

The biochar used in this study was produced from yellow pine wood by slow pyrolysis at 500°C for 30 min (AirTerra Inc., Canada). The pH of the biochar was 9.6 and contained more than 80% C on a dry weight basis (AirTerra Inc., Canada). The biochar used in this study is highly alkaline (pH = 9.6) and its ash content is mainly dominated by the macronutrients, especially Ca, Mg and K, which may be present inside the carbon matrix as different poorly crystalline minerals (Singh et al., 2010). These minerals may be responsible for the alkalinity of the material. **Table 2.1** shows the basic characteristics of biochar used in this study.

Table 2.1: Basic characteristic of biochar used in this study

Characteristic	Value
pH	9.6
Moisture content	14.9
Electrical Conductivity (EC) (dSm^{-1})	5.2
Bulk density (gcm^{-3})	0.20
Ash %	5.8
Carbon %	>80
Feed stock	Yellow Pine Wood
Particle size (mm)	1 - 6
Ca	9.82%
Mg	2.9%
K	1.12%

2.7.2 Variation of soil pH with the addition of biochar

Fly-ash used in this study contains significant quantity of trace elements. The soils used in this study were collected from uncontaminated sites; however, they naturally contained significant amounts of Ni, Cr, Fe, Mn, Zn and Cu. Heavy metal retention by biochar is discussed in literature and numerous mechanisms such as ion exchange, electrostatic attraction, physical adsorption, and carbonate precipitation are involved (Li et al., 2017). Given the alkaline nature of biochar, it is difficult to isolate the action of pH changes from other effects, especially when biochar is mixed with soil. .

Table 2.2 shows the variation of the soil pH with the addition of biochar. pH of the soil showed a significant ($p<0.05$) increase with the addition of biochar. Variation of the soil pH after leaching also showed a significant difference. The importance of the pH effect induced by biochar has been observed in the present study for the naturally acidic soils used, as the pH of the leachate (in most of the leaching events) was higher in the soil amended with biochar compared to the soil without biochar (**Figure 2.8**). This is probably due to dissolution of alkaline components from biochar.

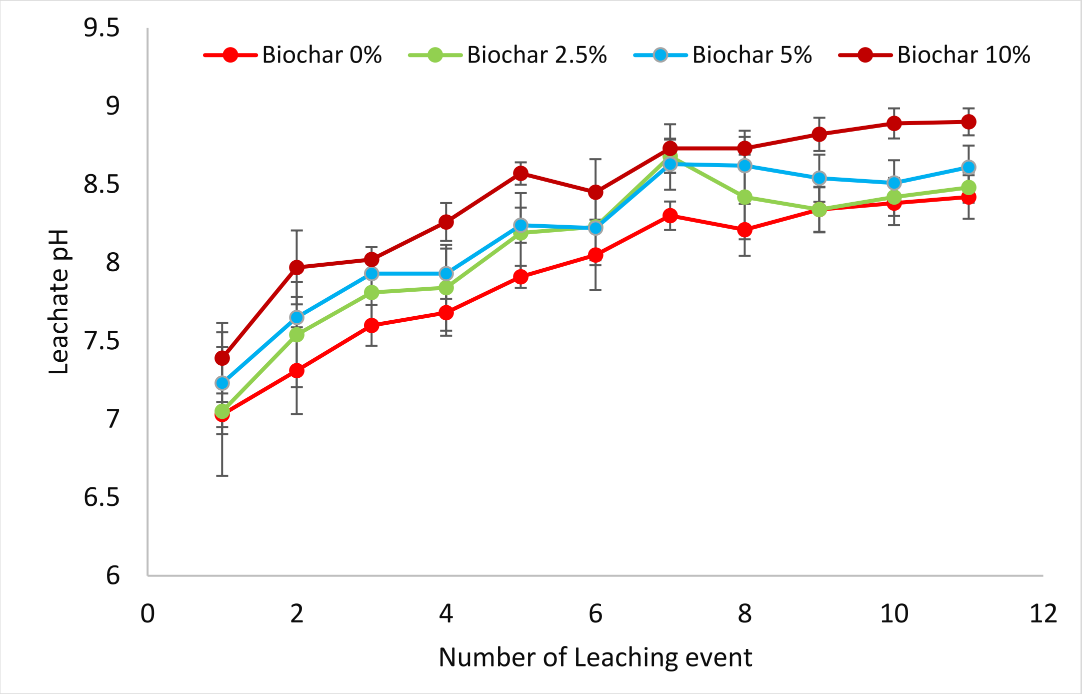


Figure 2.8: Variation of leachate pH throughout the leaching event showing the effect of addition of biochar and leaching events (one leaching event = 0.5 pore volume)

2.7.3 Variation of heavy metal concentration in the leachate during the leaching events

Biochar is quite like activated carbon with respect to mutual production via pyrolysis, with medium to high surface areas (Cao et al., 2011). However, unlike activated carbon, biochar is generally not activated or treated (Cao and Harris, 2010). Additionally, the biochar contains a non-carbonized fraction such as $CO_3^{2-}$and PO_4^{3-}that may interact with soil contaminants. Specifically, the extent of O-containing carboxyl, hydroxyl, and phenolic surface functional groups in biochar could effectively bind soil contaminants (Uchimiya et al., 2011b). These multi-functional characteristics of biochar show the potential as a very effective environmental sorbent for organic and inorganic contaminants in soil and water. Soil pH is considered to greatly influence the mobility of metals as speciation changes with pH. Generally, biochar is alkaline, thereby inducing liming effect in soil and causes immobilization of metals and mobilization of oxyanions (Almaroai et al., 2013). As discussed in section 4.2, biochar-induced increases in soil pH can also influence the sorption of metals.

Increasing the soil pH can immobilize metals in soil because of several reasons. Firstly, increasing the soil pH of variable charged soils, can increase the negative charges on the soil surface, thus increasing metal adsorption. Secondly, the increase in soil pH increases the hydrolysis of heavy metals and, in turn, increases their adsorption by the variable-charged soils because of the higher adsorption affinity by the soil surface for metal hydroxides than the unhydrolyzed metal ions. Study results show that most of the time the leached concentrations of most of the metals are the highest in control conditions (no biochar). This finding is consistent with previous studies. For instance, Rao et al. (2007) claimed that low pH generally favored the leaching of many metals from fly-ash. Release of metals under acidic conditions increases due to dissolution of metal-bearing mineral. Presence of heavy metals in leachate were observed throughout the entire leaching period and varying trends were observed for different elements.

2.7.4 Variation of Mo concentration in leachate throughout the leaching event

Molybdenum becomes more soluble and is accessible to plants mainly, as MoO_4^{2-} , in its anion form. In contrast, in acidic soils (pH <5.5) molybdenum availability decreases as anion adsorption to soil oxides increase (Reddy et al., 1997). The variation of leaching concentrations of Mo is shown in **Figure 2.9**. During the initial leaching events (First 3 leaching events) the Mo concentration in the leachate increased with the number of leaching events and no significant differences ($P<0.05$) were observed between treatments. From 4th leaching event onward significant differences were observed between the treatments. After reaching a peak, the concentration of Mo in leachate started to decrease in the biochar amended soil whereas after reaching a peak it remained as a constant value in the control soil. The highest concentration of Mo was observed in 10% (w/w) biochar amended soil with a value of 25.06 µg/L.

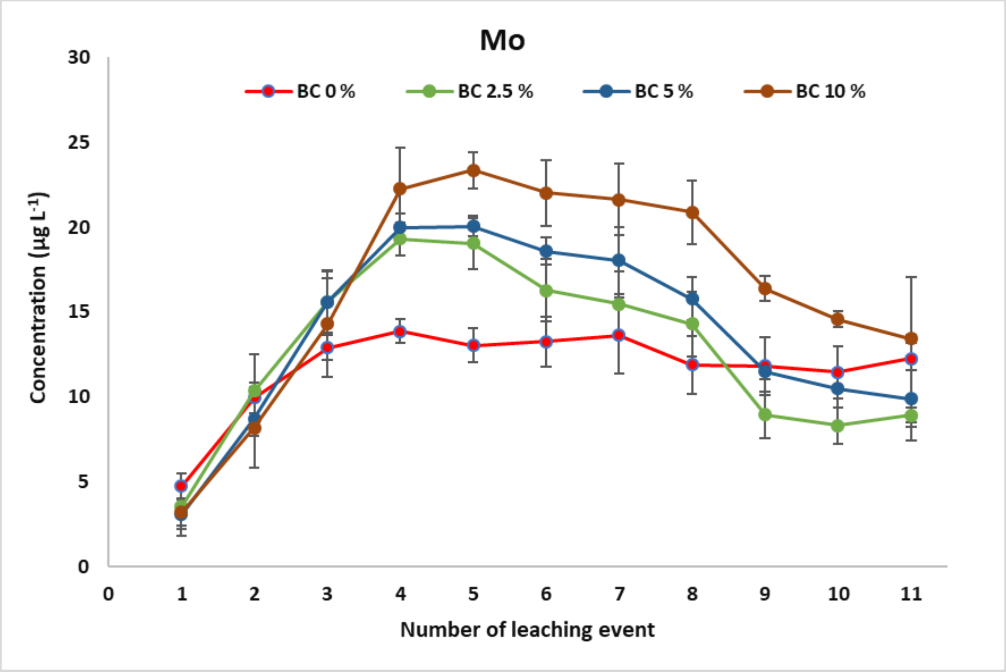


Figure 2.9: Variation of Mo concentration in leachate throughout the leaching event (one leaching event = 0.5 pore volume)

Mo leaching from the soil columns were low in the first leaching time, which may be attributed to the required time for Mo to move from the upper surface layer to the bottom of the soil column and for the Mo-bound compounds to functionalize with the soil components.

2.7.5 Variation of Fe, Ni, Cu and Zn concentration in leachate throughout the leaching event

It is well known that Fe solubility is controlled by hydroxide minerals such as hematite (Fe_2O_3) and Fe $(OH)_3$. Potential presence of these solid phases in the soil solutions has control over the leaching potential of Fe elements at different conditions. These oxide/hydroxide minerals tend to release Fe at very acidic and basic conditions which increase the leaching concentrations of Fe into effluent solutions (Komonweeraket et al., 2015). Ni solubility decreases with increasing pH in inorganic systems due to the increased sorption of Ni(II) species on negatively charged mineral surfaces (Richter and Theis1980), and the tendency of Ni(II) species [$Ni(OH)^+$ and $Ni(OH)_2$] to form hydrolysed surface complexes or precipitates when pH >9 (for concentrations $<1\times10^{-3}$M) (Bradbury and Baeyens2009; Peacock and Sherman 2007).

Cu solubility in soils is often controlled by pH. At neutral and high pH values, Cu^{2+}adsorbs strongly to negatively charged mineral surfaces, and solution concentrations are low (Peacock and Sherman 2004). Leaching of Cu is solubility controlled by the dissolution /precipitation of tenorite (CuO) and Spertiniite ($Cu(OH)_2$). Dissolution and precipitation of these solid phases are likely to influence release of Cu into aqueous solution (Komonweeraket et al., 2015).

Notably, Cu mobility/immobility is highly influenced by biochar organic C content. Generally, the biochars produced at <500°C have high dissolved organic carbon (DOC) content, which could facilitate the formation of soluble Cu complexes with DOC, as reported by Beesley et al. (2010) and Park et al. (2011). Additionally, DOC can block the pores of biochars preventing Cu sorption (Cao et al., 2011). However, the biochar produced at high temperatures are generally deficient in DOC, which could decrease Cu mobility in soil, as reported by Uchimiya et al. (2011c). The biochar used in this study was produced at 500°C and the addition of biochar decreased the Cu mobility, perhaps the biochar used in the study did not have a large enough content of DOC or in this case the more important feature was soil pH increase with the addition of biochar.

The leaching of Zn is controlled by dissolution–precipitation of the oxide and hydroxide minerals such as zincite and $Zn(OH)_2$ (Komonweeraket et al., 2015). Zn immobilisation by biochar is controlled by complexation, electrostatic attraction, and precipitation. Moreover, Ca-oxalate crystals may be responsible for the increased ability of some biochar to remove Cd and Zn (Clemente et al., 2017). Lu et al. (2014) reported that reduction in biochar particle size can enhance the effectiveness of Zn immobilization illustrating biochar size also should be taken into consideration when in use as soil amendment.

The variation of leaching concentrations of Fe, Ni, Cu and Zn throughout the leaching events are shown in **Figure 2.10**. Throughout the leaching period highest Fe, Cu and Ni concentrations were observed in 0% (w/w) biochar amended soil. The observed highest concentration of Fe, Cu, Ni and Zn were 65.91, 7.02, 6.05 and 115.8 µg/L respectively. Throughout the leaching events lowest Fe, Cu and Ni concentrations were observed in leachate from 10% (w/w) biochar amended soil. From the third leaching event concentration variation of Zn shows a similar trend in all treatments. But high concentration of Zn was observed in 0% (w/w) biochar amended soil when compared to others. In most of the leaching event, the Lowest Zn concentrations were observed in 10%(w/w) biochar amended soil. Similar trends were observed for the changes in the Cu concentration of each treatment **(Figure 2.10(C))**. Throughout the experiment period significant differences were observed in Cu concentration between the treatments. Also, similar trends were observed for the changes in the Ni concentration of each treatment **(Figure 2.10(B))**. In most of the leaching events significant differences between the treatments were observed.

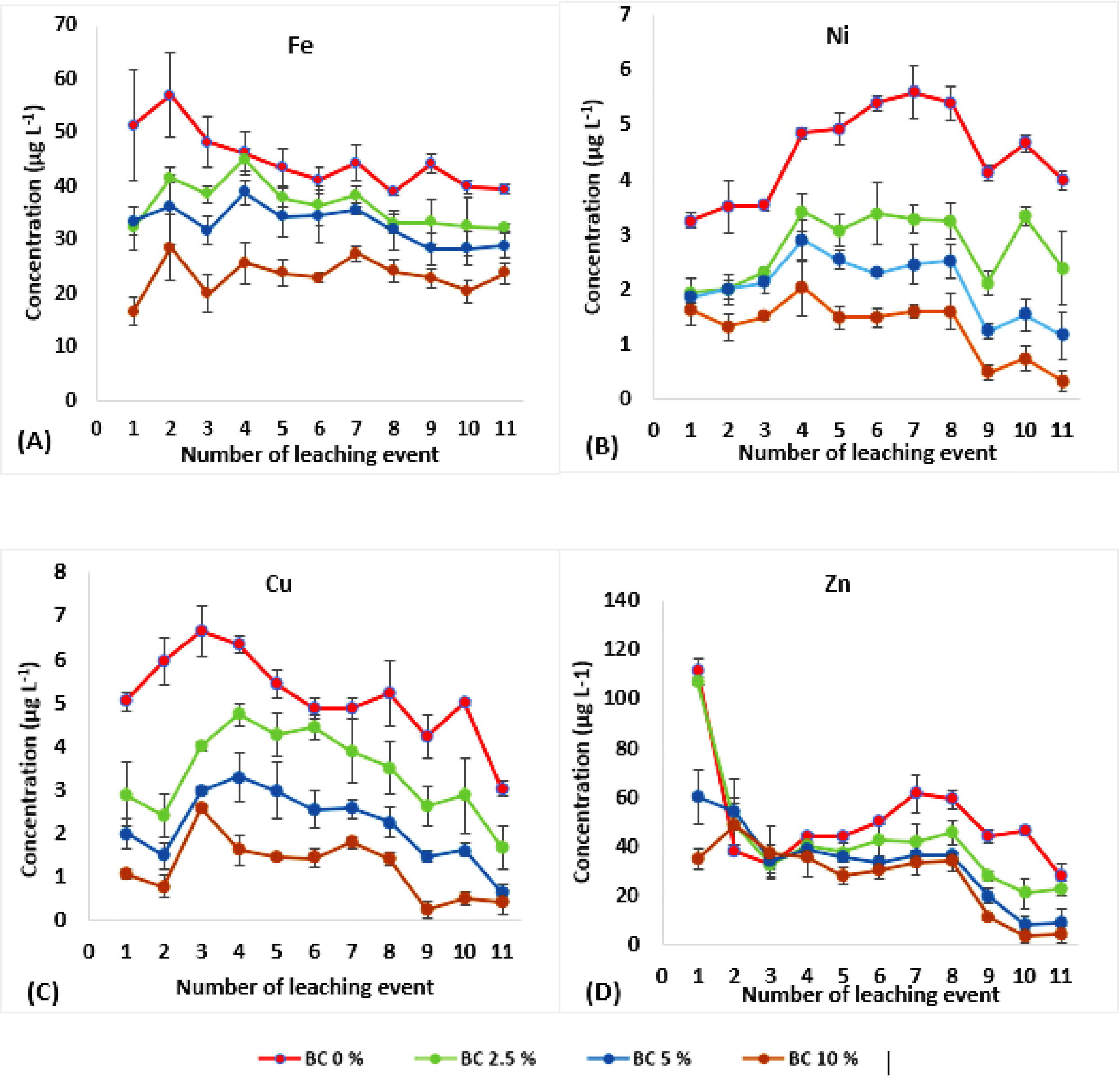


Figure 2.10: Variation of (A) Fe, (B) Ni, (C) Cu, (D) Zn concentration in leachate throughout the leaching event (one leaching event = 0.5 pore volume)

2.7.6 Variation of Cr, V, Pb and Co concentration in leachate throughout the leaching event

Leaching of Cr is highly dependent on the dissolution/precipitation reaction of Cr carrying oxide/hydroxide minerals. It is expected that the solubility of Cr metals is controlled by chromium oxide/hydroxide minerals such as Cr_2O_3(s) and $Cr(OH)_3$(s)(Komonweeraket et al., 2015). Biochar application can also reduce the leaching of metals through its effect of redox reactions of metals. For example, Choppala et al. (2012) showed that the application of biochar derived from chicken manure to chromate contaminated soils enhanced the reduction of mobile Cr(VI) to less mobile Cr(III), thereby decreasing the leaching of Cr. The decrease in the leaching of Cr(III) is attributed to the adsorption of Cr(III) onto cation exchange sites and also to the precipitation as $Cr(OH)_3$ resulting from the release of OH − ions during the Cr(VI) reduction process (Bolan et al. 2014).

Application of various extraction and leaching methods to field-contaminated soils and sediments has demonstrated that a very small fraction (generally <1%) of the vanadium is readily dissolved (Cappuyns and Swennen, 2014; Teng et al., 2011). Extremely low pH values enhance the solubility (Cappuyns and Swennen, 2014; Mikkonen and Tummavuori, 1994b). Competition with other anionic species such as phosphate and arsenate may also reduce vanadium sorption in soils (Mikkonen and Tummavuori, 1994a). Over a long-term perspective, the behaviour of vanadium in soils is less well known, but its solubility has been shown to decrease with time (Martin and Kaplan, 1998).

Cadmium and lead are divalent cations and their sorption behaviour is similar. Pb sorption is defined by the same mechanisms as sorption of Cd and depends on feedstock and pyrolysis temperature of biochar (Li et al., 2017). Increasing the soil pH can affect the precipitation of heavy metals. The minimum pH ranges for the precipitation of Cd and Pb hydroxides in the soil system were 8.8 – 9.8 and 6.1 – 9.1 for Cd and Pb, respectively (source). However, the pH range of the soil used in this study was 6.67– 7.38 **(Table 2.2**). For instance, Ahmad et al. (2013) reported that in soil amended with biochar, rise in soil pH favored the sorption of Pb onto kaolinite making charge on kaolinite more negative.

Non-electrostatic mechanisms are considered as dominating for Pb (Li et al., 2016; Clemente et al., 2017; Tang et al., 2013; Uchimiya et al., 2011). But Cao et al. (2011) reported immobilization of Pb by forming $Pb_5(PO_4)_3(OH)$ in soils amended with dairy manure biochar. Uchimiya et al. (2012) showed higher Pb immobilization performance using a low pyrolysis temperature biochar. This biochar contained O-containing functional groups playing an important role in altering of Pb mobility. Increasing soil CEC had a direct effect on increasing the adsorption of heavy metals. Several studies found that the adsorption capacity of heavy metals such as Pb had a significant correlation with soil CEC.

The variation of leaching concentrations of Cr, V, Pb and Co throughout the leaching events are shown in **Figure 2.11**. Throughout the leaching period highest Cr, V, Pb and Co concentrations were observed in 0% (w/w) biochar amended soil. The observed highest concentration of Cr, V, Pb and Co were 1.44, 3.59, 0.50 and 0.92 µg/L respectively. Throughout the leaching events lowest Cr, V, Pb and Co concentrations were observed in leachate from 10% (w/w) biochar amended soil.

Similar trends were observed for the changes in the Cr, V, Pb and Co concentration in 0 and 2.5% (w/w) biochar amended treatments. Throughout the experiment period significant differences were observed in Cr, V, Pb and Co concentration between the treatments. Also, similar trends were observed for the changes in Cr, V, Pb and Co concentration in 5 and 10% (w/w). In most of the leaching events significant differences between the treatments were observed.

During the initial leaching events the Pb and Cr concentration in the leachate was increased with the number of leaching events and there is not any significant difference in Cr and Pb concentration between treatments during the initial stage of leaching. After the third leaching events significant differences were observed between the treatments. Variation of Cr, Pb and Co concentration in the leaching from 10% biochar amended soil almost remains as constant throughout the leaching events. 0% (w/w) biochar amended soil produced a peak during the leaching event for Cr, V, Pb and Co which is the highest concentrations observed during the experiments.

Similar trends were observed for the changes in the V concentration of each treatment **(Figure 2.11(B))**. Cr, Pb and Co concentrations in leachate increased with the leaching events and reached a peak. The application of water produced a pulse in the leachates during the experiment, then concentrations decreased thereafter. In most of the leaching events significant difference between the treatments were observed. Statistical analysis results showed that the concentration of the Cr, Pb, V and Co leached from the soil columns were significantly affected by biochar incorporation.

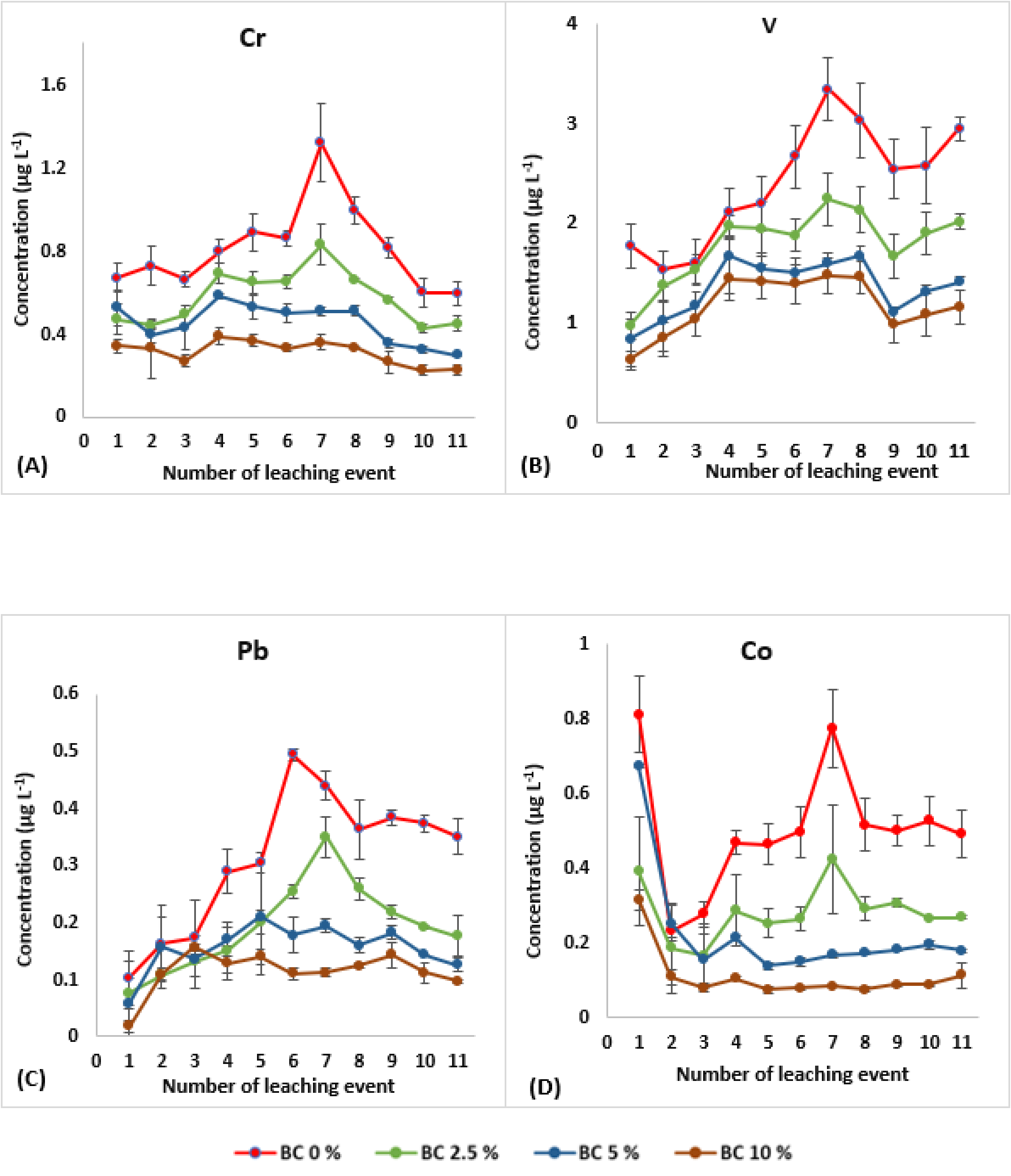


Figure 2.11: Variation of (A)Cr, (B) V, (C) Pb, (D) Co concentration in leachate throughout the leaching event (one leaching event = 0.5 pore volume)

2.7.7 Variation of As concentration in leachate throughout the leaching event

Arsenic is a soil contaminant that is given special attention due to its toxicity. Unlike cationic metals, it is present as an oxy anion in solution and presents some specific challenges to remediation because, unlike metals, arsenic mobility in soil is increased with increasing soil pH and binds to anion exchange sites on soils. This may also mean that those material conditions induced by biochar addition to soils, which may not necessarily impact on metal mobility, could control As mobility, regardless of the capacity of biochar as a sorbent.

Biochar produced at lower temperature is potentially more efficient in As removal (Li et al., 2017). However, in some cases biochar application does not induce As immobilization. Arsenic is attracted by positively charged sites in soil, but after the biochar application pH increases and amount of these sites becomes lower. Another possible mechanism increasing its mobility in the amended soils is electrostatic repulsion with biochar surface (Igalavithana et al., 2017).

There has been a significant increase during the last decade to investigate effectiveness of biochar as a soil amendment to remediate As-contaminated soils (Steiner et al., 2007; Lehmann et al., 2003). Upon application of biochar to soils, porous organic-inorganic layers establish on the soil surface, and these layers have a higher number of functional groups that create more adsorption sites for As (Bian et al., 2014). Also, biochar might affect behaviour of As in soils via its impact on soil pH, CEC, SOM, and other physicochemical and microbial properties (Vithanage et al., 2017). Biochar may have demonstrated effectiveness for As removal from wastewaters (Mohan and Pittman, 2007) but some concerns surround their application to As contaminated soils due to the potential increases in soil pH (Hartley et al., 2009) and soluble C (Beesley et al., 2010).

The variation of leaching concentrations of As is shown in **Figure 2.12.** These results highlight that biochar was able to act as an immobilizing agent to reduce concentrations of As. In most of the leaching events significant difference between the control soil and biochar amended soil were observed. Throughout the leaching period higher As concentrations were observed in 0% (w/w) biochar amended soil and highest was 9.26 µg/L. there were no any significant difference between 5% and 10% biochar amended soil.

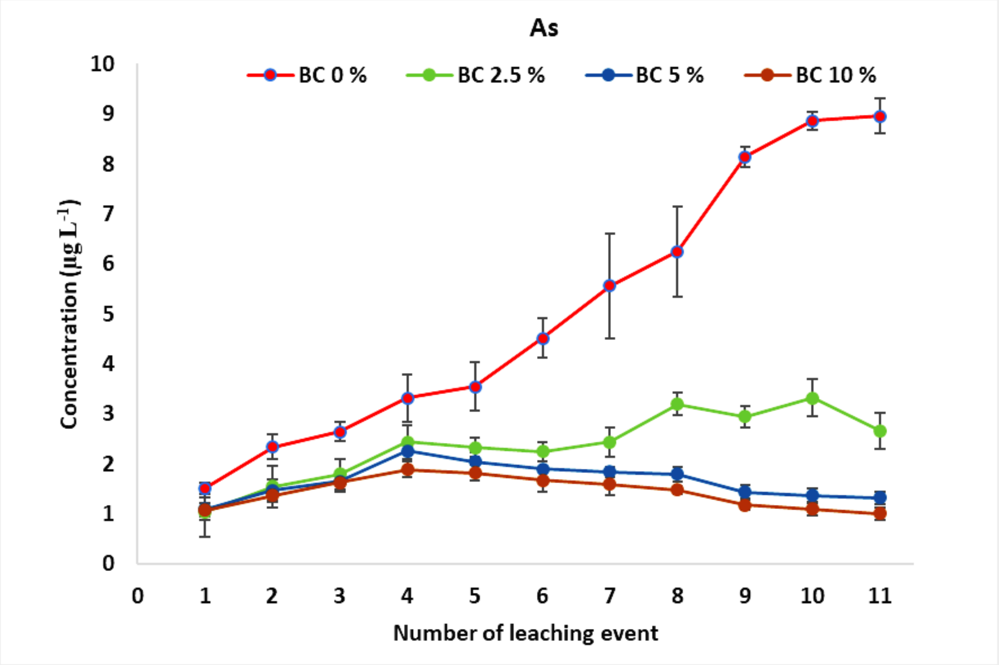


Figure 2.12: Variation of As concentration in leachate throughout the leaching event (one leaching event = 0.5 pore volume)

2.7.8 Summary of metal concentration in leachate throughout leaching event

According to the results, in most of the leaching events biochar amendment significantly enhanced the metal retention except for Mo. Variation of Hg and Cd concentration in leachate throughout the leaching event were not discussed because most of their concentrations in the leachate were below the detection limits.

Observed maximum concentrations of each heavy metal were compared with different Canadian water quality guidelines such as Guidelines for Canadian Drinking Water Quality, Water Quality

Guidelines for the Protection of Agriculture (Irrigation and livestock) and Fresh Water Quality Guidelines for the Protection of Aquatic Life. **Table 2.2** shows the maximum observed metal concentration in leachate during leaching event and different quality guidelines.

According to the result, the maximum metal concentrations were still below the quality standards for drinking water, thereby suggesting that leaching from the fly-ash amended soil used in this study is unlikely to cause contamination to ground water.

Table 2.2. Maximum observed metal concentration in leachate during leaching event and different quality guidelines. Comparisons show that the leachate concentrations after 11 leaching events are well below the guidelines provided by the Canadian drinking water and agriculture water qualities (all values in µg L^{-1})

<table>
<tr><th colspan="12">Maximum observed metal concentration in leachate during leaching event</th></tr>
<tr><th>Biochar
(% w/w)</th><th>V</th><th>Cr</th><th>Fe</th><th>Ni</th><th>Co</th><th>Cu</th><th>Zn</th><th>As</th><th>Mo</th><th>Cd</th><th>Pb</th></tr>
<tr><td>0</td><td>3.59</td><td>1.44</td><td>65.91</td><td>6.05</td><td>0.92</td><td>7.02</td><td>115.8</td><td>9.26</td><td>16.80</td><td>0.79</td><td>0.50</td></tr>
<tr><td>2.5</td><td>2.43</td><td>0.91</td><td>46.98</td><td>4.0</td><td>0.59</td><td>5.03</td><td>108.7</td><td>3.69</td><td>20.68</td><td>0.50</td><td>0.37</td></tr>
<tr><td>5</td><td>1.77</td><td>0.62</td><td>41.34</td><td>3.30</td><td>0.68</td><td>3.93</td><td>69.15</td><td>2.46</td><td>20.78</td><td>0.42</td><td>0.33</td></tr>
<tr><td>10</td><td>1.68</td><td>0.49</td><td>34.55</td><td>2.62</td><td>0.34</td><td>2.62</td><td>59.39</td><td>2.07</td><td>25.06</td><td>0.33</td><td>0.17</td></tr>
<tr><th colspan="12">Guidelines for Canadian Drinking Water Quality</th></tr>
<tr><td></td><td>-</td><td>50</td><td>300</td><td>-</td><td>-</td><td>1000</td><td>5000</td><td>10</td><td>-</td><td>5</td><td>10</td></tr>
<tr><th colspan="12">Water Quality Guidelines for the Protection of Agriculture</th></tr>
<tr><td></td><td>V</td><td>Cr</td><td>Fe</td><td>Ni</td><td>Co</td><td>Cu</td><td>Zn</td><td>As</td><td>Mo</td><td>Cd</td><td>Pb</td></tr>
<tr><td>Irrigation</td><td>100</td><td>8</td><td>5000</td><td>200</td><td>50</td><td></td><td></td><td>100</td><td></td><td>5.1</td><td>200</td></tr>
<tr><td>Livestock</td><td>100</td><td>50</td><td></td><td>1000</td><td>1000</td><td></td><td>50000</td><td>25</td><td>500</td><td>80</td><td>100</td></tr>
</table>

2.8 Metal contents in the leachate and soil column

The accumulated mass (μg) of each metal in the leachate varied depending on the treatment applied. In the soil column experiment the amount of metals leached was the highest in the absence of biochar, except for Mo. The addition of biochar to the soil reduced the metal concentrations present in the leachate in general. The reduction in the amount of leachable metals in soil amended with biochar in comparison with the control can be attributed to several factors, including the increasing pH and CEC of the soil. This increase in pH and CEC of the soil may have increased the metal adsorption because of the increase in adsorption sites (Fahimi, 2018). Similarly, according to Bashir et al., the reduction of heavy metals extracted from contaminated soil after the addition of biochar could be due to the increase in soil pH, which would increase the immobilization of heavy metals through adsorption and precipitation. Moreover, a higher pH promotes the adsorption of metals on biochar, as the density of the negative charge also increases on the biochar surface. Inyang et al. reported that the CEC of plant material biochar was controlled by its functional groups content. Heavy metals could be complexed with biochar functional groups. Uchimiya et al. (2010) found that the retention of heavy metals in soil by surface ligands was strongly pH dependent.

Figure 2.13 shows the accumulated mass of metals leached from each column after 11 leaching events. The addition of biochar to the soil reduced the amount of metal leached from the soil except Mo, which showed the opposite trend of increasing amounts leached with biochar additions.

An analysis of the total leaching amounts of heavy metals can directly reflect the leaching strength of the said heavy metals. The total leaching amounts of Hg and Cd were not calculated because most of their concentrations in the leachate were below the detection limits.

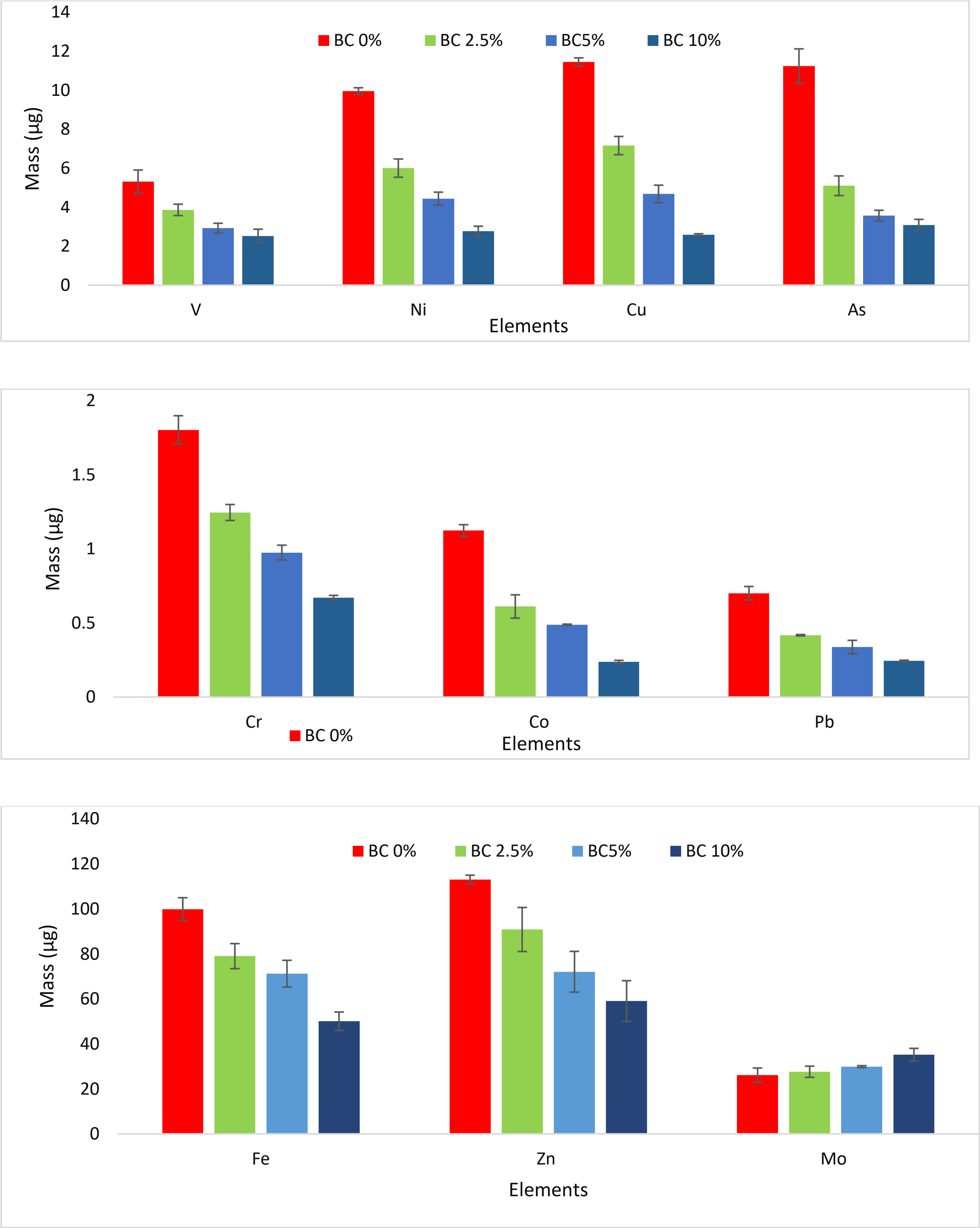


Figure 2.13: Total amount of metals leached from the columns after 11 leaching events

Table 2.4. The concentrations of the heavy metals in fly-ash amended soil before leaching. Mean values (mg kg^{-1}) are presented with standard errors (n=3) and the amount of heavy metal leached (%) from soil column (nd = not determined)

Element	Concentration (mg kg^{-1})	Biochar 0%	Biochar 2.5%	Biochar 5%	Biochar 10%
V	29.82 ± 2.16	0.012	0.009	0.007	0.006
Cr	21.14 ± 0.96	0.006	0.004	0.004	0.002
Fe	111.9 ± 3.21	0.072	0.061	0.054	0.036
Ni	24.06 ± 2.91	0.029	0.018	0.015	0.010
Co	6.04 ± 0.68	0.013	0.007	0.006	0.004
Cu	12.96 ± 0.94	0.069	0.046	0.030	0.018
Zn	4.01 ± 0.35	0.099	0.089	0.075	0.062
As	4.91 ± 0.76	0.150	0.079	0.069	0.061
Mo	60.9 ± 2.21	0.031	0.039	0.042	0.051
Pb	10.01 ± 0.76	0.005	0.003	0.002	0.002
Cd	0.51 ± 0.08	nd	nd	nd	nd
Hg	0.23 ± 0.12	nd	nd	nd	nd

In general, water-soluble fraction of heavy metal in fly-ash and fly-ash amended soil is very low (Kumar et al., 2017: Zheng et al., 2012). My study showed that the total amount of heavy metal leached from all treatments is very low compared to the total retained heavy metal **(Table 2.4)**. However, there is a difference between the treatments. For all elements, except for Mo, 10% biochar amended soil column showed least fraction of heavy metal leachate compared to others

Table 2.5 Heavy metal concentration in each soil layer after leaching events. Mean values (mg kg^{-1}) are presented with standard errors (n=3)

Biochar %	Soil Layer	V	Cr	Ni	Cu	Zn	As	Cd	Co	Pb
0	0 - 10	27.02 ± 0.80	19.88 ± 0.16	22.91 ± 1.34	11.75 ± 0.29	88.14 ± 3.16	3.62 ± 0.19	0.31 ± 0.02	5.89 ± 0.01	9.01 ± 0.24
0	10 - 20	27.59 ± 0.53	19.90 ± 0.16	23.24 ± 0.93	11.85 ± 0.29	89.06 ± 3.13	3.63 ± 0.15	0.33 ± 0.03	5.86 ± 0.03	9.02 ± 0.26
0	20 - 30	27.71 ± 0.55	19.90 ± 0.16	23.39 ± 0.95	11.87 ± 0.28	89.13 ± 3.15	3.66 ± 0.18	0.32 ± 0.02	5.89 ± 0.01	9.03 ± 0.25
2.5	0 - 10	27.60 ± 0.55	19.89 ± 0.16	23.36 ± 0.90	11.85 ± 0.27	88.60 ± 3.16	3.64 ± 0.18	0.34 ± 0.01	5.89 ± 0.01	9.20 ± 0.39
2.5	10 - 20	27.67 ± 0.51	19.93 ± 0.17	23.27 ± 0.95	11.94 ± 0.21	89.76 ± 3.18	3.65 ± 0.18	0.34 ± 0.01	5.86 ± 0.04	9.02 ± 0.25
2.5	20 - 30	27.79 ± 0.48	19.96 ± 0.13	23.25 ± 0.92	11.87 ± 0.30	89.04 ±3.17	3.65 ± 0.18	0.33 ± 0.01	5.89 ± 0.01	9.03 ± 0.25
5	0 - 10	27.62 ± 0.52	19.90 ± 0.16	23.27 ± 0.82	11.93 ± 0.22	89.67 ±3.17	3.66 ± 0.18	0.33 ± 0.02	5.89 ± 0.01	9.02 ± 0.25
5	10 - 20	27.61 ± 0.53	19.91 ± 0.13	23.26 ± 0.90	11.91 ± 0.31	89.09 ± 3.15	3.65 ± 0.18	0.34 ± 0.02	5.82 ± 0.03	9.02 ± 0.25
5	20 - 30	27.60 ± 0.53	19.94 ± 0.13	23.28 ± 0.90	11.87 ± 0.31	89.72 ± 3.15	3.66 ± 0.18	0.34 ± 0.02	5.95 ± 0.03	9.03 ± 0.25
10	0 - 10	27.65 ± 0.52	19.92 ± 0.15	23.11 ± 0.81	11.85 ± 0.30	89.64 ± 3.20	3.65 ± 0.18	0.33 ± 0.02	5.73 ± 0.25	9.01 ± 0.27
10	10 - 20	27.61 ± 0.53	19.93 ± 0.15	23.22 ± 0.96	11.83 ± 0.31	89.98 ± 2.95	3.66 ± 0.18	0.34 ± 0.01	5.93 ± 0.02	9.03 ± 0.26
10	20 - 30	27.60 ± 0.57	19.93 ± 0.15	23.30 ± 0.84	11.84 ±0.32	89.84 ± 3.15	3.66 ± 0.18	0.34 ± 0.00	5.96 ± 0.02	9.02 ± 0.25

Analysis of heavy metal concentration in each soil layer after the 11 leaching events (**Table 2.5)** showed that there is no significant difference ($p<0.05$) in heavy metal concentration within the treatments and between treatments. It may be due to the insignificant heavy metal leached (compared to total heavy metal content in soil column **(Table 2.4)** from all soil column.

2.9 Pot experiment

2.9.1 Dry biomass yield

The impact of biochar on biomass (crop yield) is very complex. To date, the effects of biochar application on crop yield show mixed result and highly variable; for example, biochar has been reported to increase crop yields (Koyama et al., 2016). However, Rajkovich (2012) reported that the effect of biochar on crop yields was negative. These highly diverse results are not surprising because of the many variables involved in studying the effects of biochar application on a cropping yield such as the characteristics and rate of the biochar used, soil type, crop species, climate condition, and other factors (Win et al., 2019). Biochar is effective in changing the physical, chemical, and biological properties of soil (Głąb et al., 2016; Sohi et al., 2010; Lehmann et al., 2011), which can increase growth and yield of crop. Some researchers observed that the addition of biochar significantly increased total C and the C/N ratio in the rhizosphere (Koyama et al., 2016; Dong et al., 2015).

In the present study, *Trifolium pratense* and *Phleum pratense* L showed positive effects on above ground biomass production with biochar application. The above ground biomass yields significantly ($p < 0.05$) increased with biochar in both plants (**Table 2.6**). The average biomass yields in the 0, 2.5, 5, and 10% biochar amended soils were 4.33, 4.85, 5.46, and 5.59 g pot^{-1}, respectively, in *Trifolium pratense*, whereas they were 3.95, 4.05, 4.30, and 4.57 g pot^{-1}, respectively, in *Phleum pratense* L.

Table 2.6. Above ground dry biomass yield (g pot^{-1}) of *Trifolium pratense* and *Phleum pratense* L under different biochar treatments (% w/w). Mean values are presented with standard errors, minimum and maximum. Means values with same letter are not significantly *($p < 0.05$)* different from each other between different biochar rates.

Plants	Biochar	Mean	Std. Error	Minimum	Maximum
Trifolium pratense	.00	4.33 (a)	0.20	4.03	4.72
	2.50	4.85 (b)	0.05	4.75	4.96
	5.00	5.46 (cd)	0.11	5.24	5.61
	10.00	5.59 (d)	0.11	5.38	5.78
Phleum pratense L	.00	3.95 (a)	0.06	3.85	4.08
	2.50	4.05 (ab)	0.05	3.95	4.12
	5.00	4.30 (bc)	0.08	4.13	4.43
	10.00	4.57 (c)	0.11	4.35	4.73

In *Trifolium pratense*, with 2.5, 5, 10% biochar treatments significantly ($p < 0.05$) increased biomass yield by 12%, 26% and 29%, respectively compared with control or 0% biochar treatment. Meanwhile, *Phleum pratense* L, with 2.5, 5, 10% biochar treatments increased biomass yield by 2%, 9 and 15%, respectively compared with control or 0% biochar treatment. Although *Phleum pratense* L treated with 2.5% biochar showed numerically increased biomass yield compared with control, but it did not reach a significant level. However, biomass production was significantly ($p < 0.05$) increased in 5% and 10% biochar treatments compared with control.

In the present study, both plants showed positive effects on root biomass production with biochar application. The root biomass yields significantly ($p < 0.05$) increased with biochar in both plants. The root biomass yields in the 0, 2.5, 5, and 10% biochar amended soils were 0.72, 0.80, 0.91, and

0.93 g pot^{-1}, respectively, in *Trifolium pratense*, whereas 0.57, 0.58, 0.61, and 0.65 g pot^{-1}, respectively, in *Phleum pratense* L (**Table 2.7**). The highest root biomass yield (0.93 g/pot) was observed for *Trifolium pratense* rather than that for *Phleum pratense L* (0.65 g/pot).

In *Trifolium pratense*, with 2.5, 5 and 10% biochar treatments significantly ($p <0.05$) increased root biomass yield by 12, 26 and 29%, respectively compared with control or 0% biochar treatment. Meanwhile, *Phleum pratense* L, with 2.5, 5 and 10% biochar treatments increased root biomass yield by 2, 9 and 15%, respectively compared with control or 0% biochar treatment.

Although *Trifolium pratense* treated with 2.5 and 5% biochar showed numerically increased root biomass yield among them, but they were not significantly different. However, root biomass production was significantly ($p <0.05$) increased in 2.5, 5 and 10% biochar treatments compared with control.

Table 2.7. Dry root biomass yield (g pot^{-1}) of *Trifolium pratense* and *Phleum pratense L* under different % biochar treatments (% w/w). Mean values are presented with standard errors, minimum and maximum. Means values with same letter are not significantly *($p < 0.05$)* different from each other between different biochar rates.

Plants	Biochar	Mean	Std. Error	Minimum	Maximum
Trifolium pratense	0.00	0.72(a)	.0338	0.67	0.79
	2.50	0.80(b)	.0099	0.79	0.83
	5.00	0.91(cd)	.0189	0.87	0.94
	10.00	0.93(d)	.0193	0.90	0.96
Phleum pratense L	0.00	0.57(a)	.009	0.55	0.58
	2.50	0.58(b)	.007	0.56	0.59
	5.00	0.61(bc)	.012	0.59	0.63
	10.00	0.65(c)	.016	0.62	0.68

2.9.2 Heavy metal accumulation in plant biomass

Metal uptake by plants can be affected by several factors including metal concentrations in soils, soil pH, CEC, organic matter content, types and varieties of plants, and plant age as well as the physiological properties of the crops and it is generally accepted that the metal concentration in soil is the dominant factor (Alloway, 1995; Adriano, 1986). Soils contaminated with trace metals may pose direct threats *i.e.* through negative effects of metals on crop growth and yield, and indirect threats *i.e.* by entering the human food chain with a potentially negative impact on human health. Even a reduction of crop yield by a few percent could lead to a significant long-term loss in production and income (Wuana and Okieimen, 2011). Some food importers are now specifying acceptable maximum contents of metals in food, which might limit the possibility for the farmers to export their crops with ash/biochar amendment if they exceed the maximum limits (Wuana and Okieimen, 2011). The concentrations of metals in plant tissues showed different results among treatments.

Zinc is one of micronutrients essential for normal plant growth, but only a small amount of Zn is required (25~150 $\mu g\ g^{-1}$ in dry tissue) (Alloway,1995). In the present study, both plants showed positive effects on reducing Zn accumulation in plant biomass with biochar application.

The Zn accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The Zn accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 15.0, 12.9, 7.4, and 4.2 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 9.9, 8.0, 5.6, and 3.6 mg kg^{-1}, respectively, in *Phleum pratense* L.

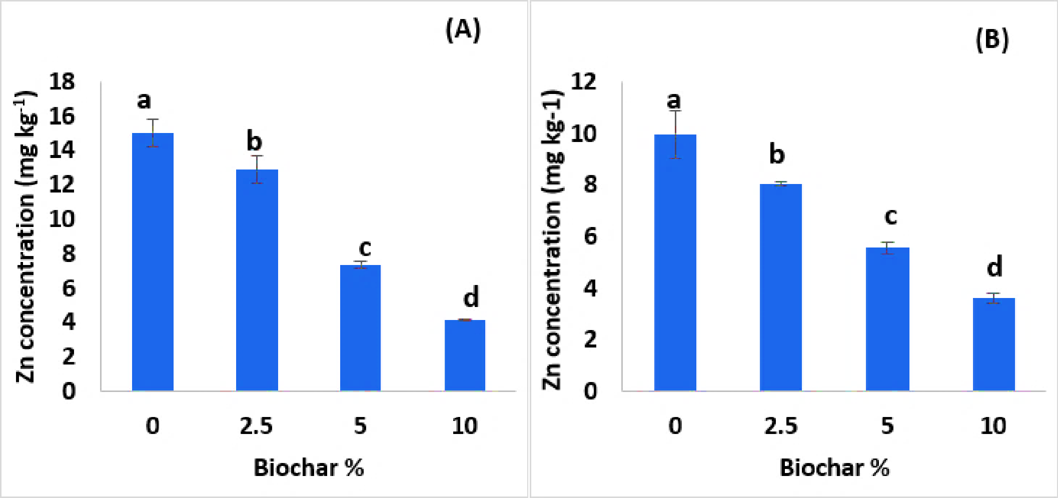


Figure 2.14: Zn concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

In *Trifolium pratense*, with 2.5, 5, 10% biochar treatments significantly ($p < 0.05$) reduced Zn accumulation by 14, 51 and 72%, respectively **(Figure 2.14)** compared with control or 0% biochar treatment. Meanwhile, *Phleum pratense* L, with 2.5, 5, 10% biochar treatments significantly ($p < 0.05$) reduced Zn accumulation by 13, 29 and 64%, respectively compared with control or 0% biochar treatment.

Although Cu is essential for plant growth, a very small amount of Cu is required by plants, for example, 5 to 20 µg g^{-1} (DW) in plant tissue (Adriano, 1986). However, over 20 µg g^{-1} (DW) can be found in plants from contaminated area, especially plant roots grown in mining and smelting sites (Jung and Thornton, 1997; Adriano, 1986; Alloway, 1995).

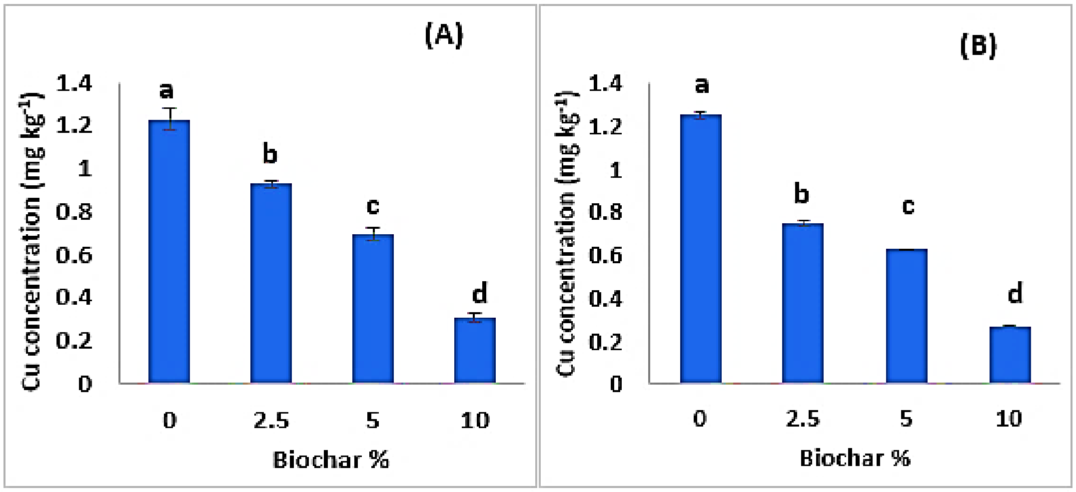


Figure 2.15: Cu concentration in (A) *Trifolium pratense* (B) *Phleum pratense L* (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Results indicated that the addition of biochar in different application rates reduced the concentration of Cu in plant biomass. The Cu concentration in the 0, 2.5, 5, and 10% biochar amended soils were 1.2, 0.9, 0.7, and 0.3 mg kg^{-1}, **(Figure 2.15)** respectively, in *Trifolium pratense*, whereas 1.3, 0.8, 0.6, and 0.3 mg kg^{-1}, respectively, in *Phleum pratense* L. In *Trifolium pratense*, when compared to the control, plant accumulation of Cu decreased by 24, 43, and 75% in 2.5, 5, and 10% biochar amended soils, respectively. whereas 40, 50 and 78%, respectively, in *Phleum pratense* L. Considering the best efficiency in reduction of Cu uptake by plant, the application 10% biochar was observed in both plants.

Pb is not an essential element. It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals. Plant Pb content is generally very low due to its low bioavailability. Lead concentrations in various plants range from 0.01 to 3.85 µg g^{-1} (DW), with an average value of 0.05 µg g^{-1} (DW) (Fergusson, 1990). In general, plants do not absorb or accumulate lead. However, in soils testing high in lead, it is possible for some lead to be taken up. Studies have shown that all lead does not readily accumulate in the fruiting parts of vegetable and fruit crops (e.g., corn, beans, squash, tomatoes, strawberries, and apples). Higher concentrations are more likely to be found in leafy vegetables (e.g., lettuce) and on the surface of root crops (e.g., carrots). Since plants do not take up large quantities of soil Pb, the Pb levels in soil considered safe for plants will be much higher than soil lead levels where eating of soil is a concern (pica) (Wuana & Okieimen, 2011). Generally, it has been considered safe to use garden produce grown in soils with total lead levels less than 300 ppm. The risk of Pb poisoning through the food chain increases as the soil Pb level rises above this concentration. Even at soil levels above 300 ppm, most of the risk is from Pb contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant (Wuana & Okieimen, 2011).

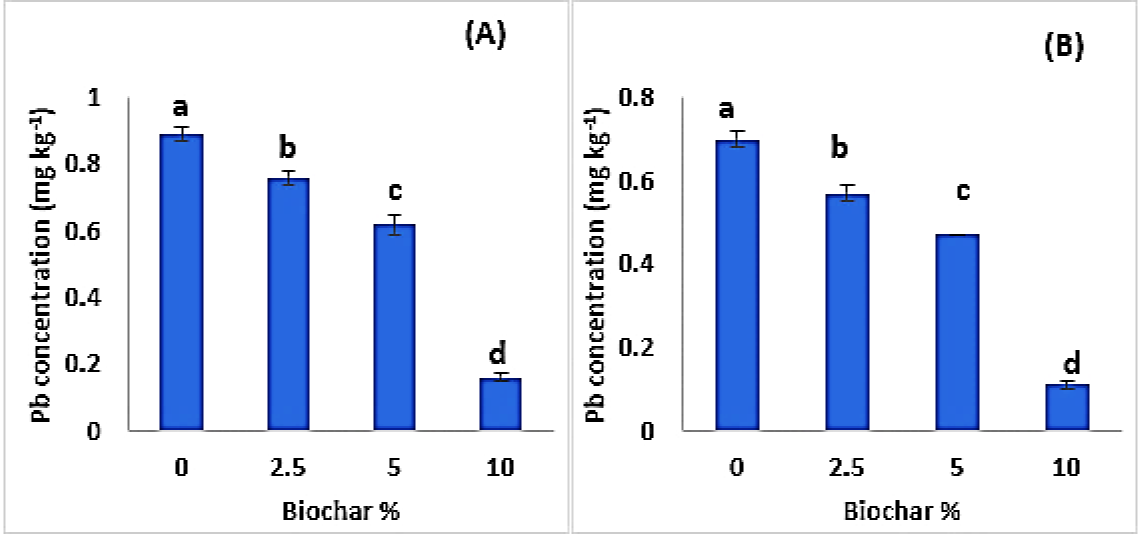


Figure 2.16: Pb concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

The Pb accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The Pb accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 0.89, 0.76, 0.62, and 0.16 mg kg^{-1}, respectively, in *Trifolium pratense* **(Figure 2.16)** whereas 0.70, 0.57, 0.47, and 0.11 mg kg^{-1}, respectively, in *Phleum pratense* L**.**

In *Trifolium pratense*, with 2.5, 5 and 10% biochar treatments significantly ($p < 0.05$) reduced Pb accumulation by 15, 30 and 82%, respectively compared with control or 0% biochar treatment. Meanwhile, *Phleum pratense* L, with 2.5, 5 and10% biochar treatments significantly ($p < 0.05$) reduced Pb accumulation by 19, 33 and 84%, respectively compared with control or 0% biochar treatment.

Nickel is an element that occurs in the environment only at very low levels and is essential in small doses, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries (Wuana & Okieimen, 2011).

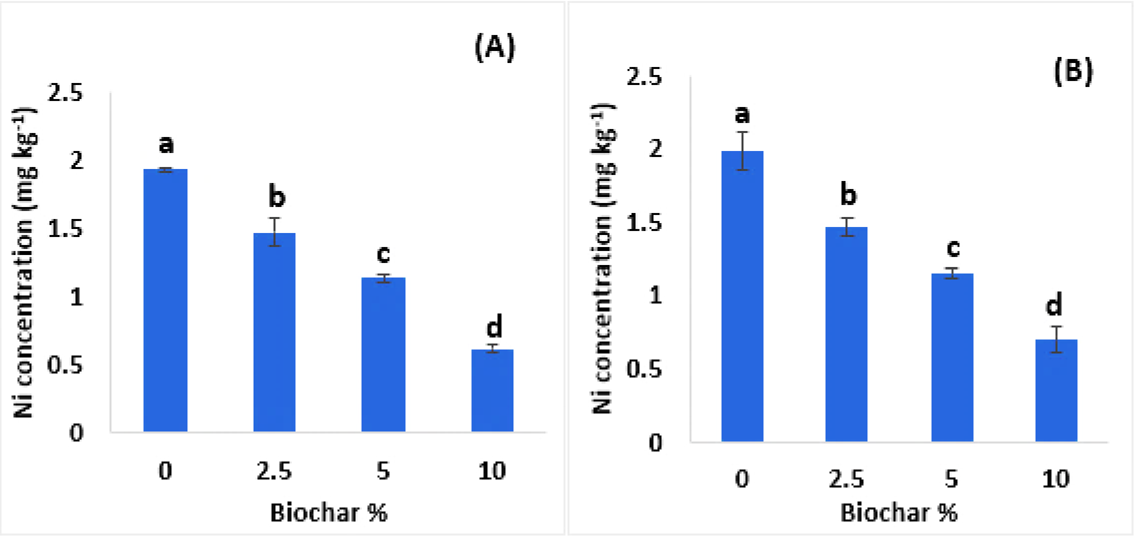


Figure 2.17: Ni concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Results indicated that the addition of biochar in different application rates reduced the concentration of Ni in plant biomass. The Ni concentration in the 0, 2.5, 5, and 10% biochar amended soils were 1.93, 1.47, 1.14, and 0.62 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 1.98, 1.46, 1.15, and 0.70 mg kg^{-1}, respectively, in *Phleum pratense* L. In *Trifolium pratense*, when compare to the control, plant accumulation of Ni decreased by 24, 41, and 68% in 2.5, 5, and 10% biochar amended soils, respectively. whereas 26, 42 and 65%, respectively, in *Phleum pratense* L **(Figure 2.17).** Considering the best efficiency in reduction of Ni uptake by plant, the application 10% biochar was observed in both plants.

Cr is not an essential element for plants ((Batish and Kohli, 2013; Huffman and Allaway 1973), yet its solubility, particularly of Cr(VI), in water is a threat for biota (Neiboer and Richardson 1980). Although Cr can enhance growth of certain plant species at lower concentrations (Shanker et al., 2009), it is highly toxic at higher concentrations and inhibits various activities in plants and may even lead to their complete damage (Batish & Kohli, 2013; Dube et al. 2003).

The Cr accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The Cr accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 2.31, 1.58, 1.08, and 0.33 mg kg^{-1}, respectively, in *Trifolium pratense* **(Figure 2.18).** whereas 1.79, 1.59, 0.87, and 0.21 mg kg^{-1}, respectively, in *Phleum pratense* L.

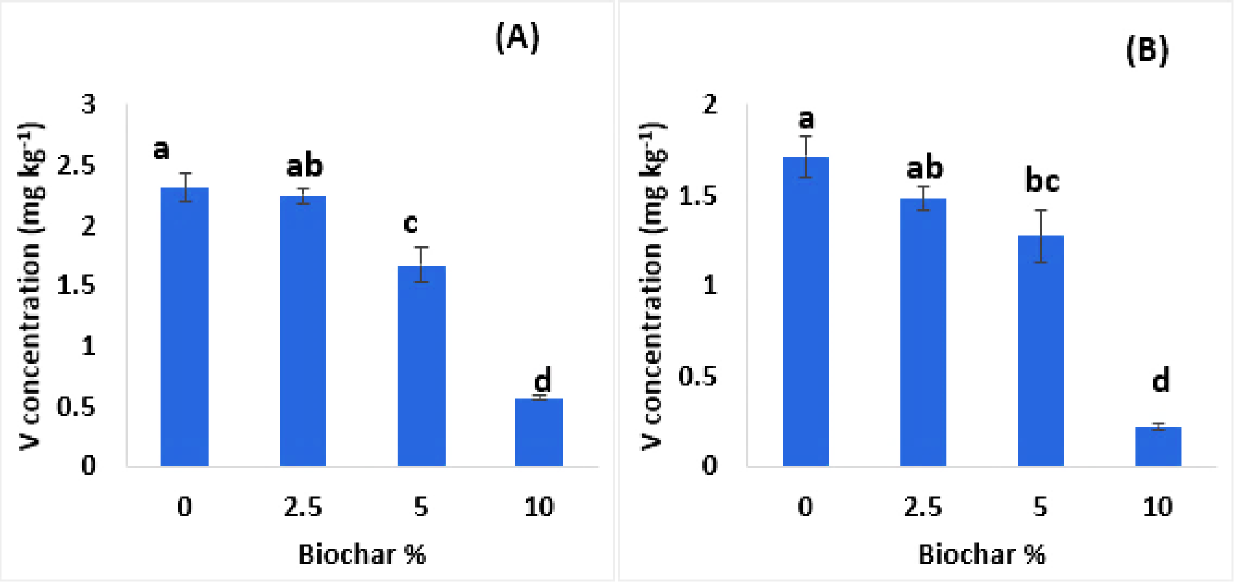


Figure 2.18: Cr concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

In *Trifolium pratense*, with 2.5, 5, 10% biochar treatments significantly ($p < 0.05$) reduced Cr accumulation by 32, 53 and 86%, respectively compared with control or 0% biochar treatment. Meanwhile, *Phleum pratense* L, with 2.5, 5, 10% biochar treatments reduced Cr accumulation by 11, 51 and 88%, respectively compared with control or 0% biochar treatment. In *Phleum pratense* L treated with 2.5% biochar showed numerically lower Cr accumulation compared with control, but the different did not reach a significant level **(Figure 2.18)**.. however, Cr accumulation was significantly ($p < 0.05$) reduced in 5% and 10% biochar treatments compared with control.

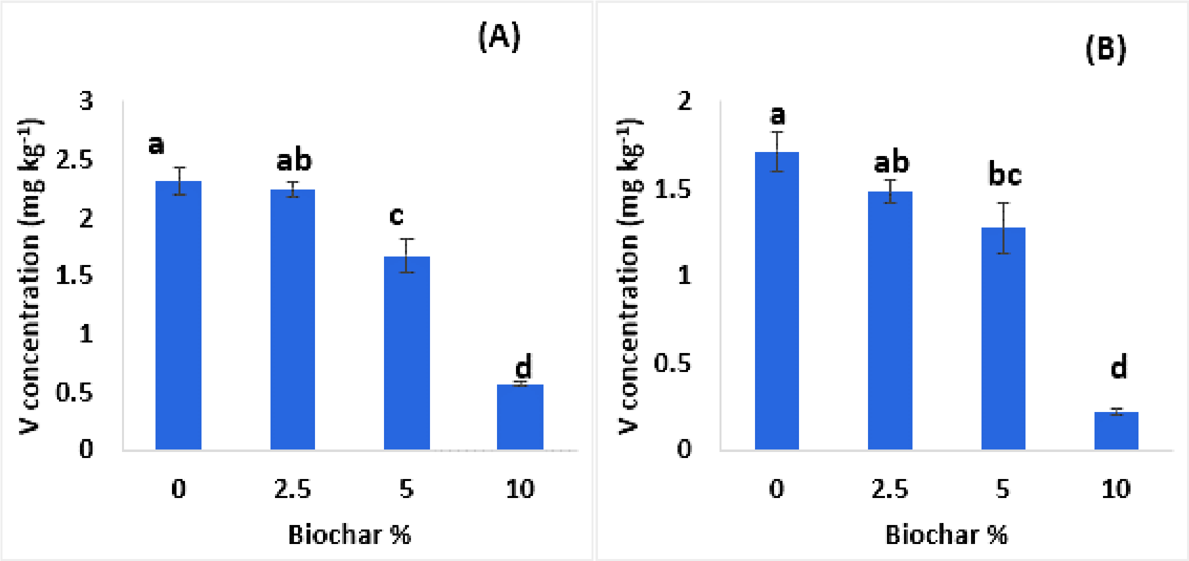


Figure 2.19: V concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Vanadium is distributed extensively in nature. In most plant species, vanadium toxicity appears at concentrations of 10 to 20 mg L $^{-1}$ and causes stunted growth (Vachirapatama et al., 2011) and chlorosis (Rosso et al., 2005). Vanadium compounds are acutely toxic by most routes of exposure, in most species. In general, the toxicity of vanadium compounds increases with the Oxidation State.

Results indicated that the addition of biochar in different application rates reduced the concentration of V in plant biomass. The V concentration in plant matter in the 0, 2.5, 5, and 10% biochar amended soils were 2.31, 2.24, 1.67, and 0.57 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 1.71, 1.48, 1.27, and 0.22 mg kg^{-1}, respectively, in *Phleum pratense* L **(Figure 2.19).** In *Trifolium pratense*, when compare to the control, plant accumulation of V decreased by 3, 28, and 75% in 2.5, 5, and 10% biochar amended soils, respectively. whereas 13, 26 and 87%, respectively, in *Phleum pratense* L. Considering the best efficiency in reduction of V uptake by plant, the application 10% biochar was observed in both plants. The 2.5% biochar did not show any significant difference with control for either plant.

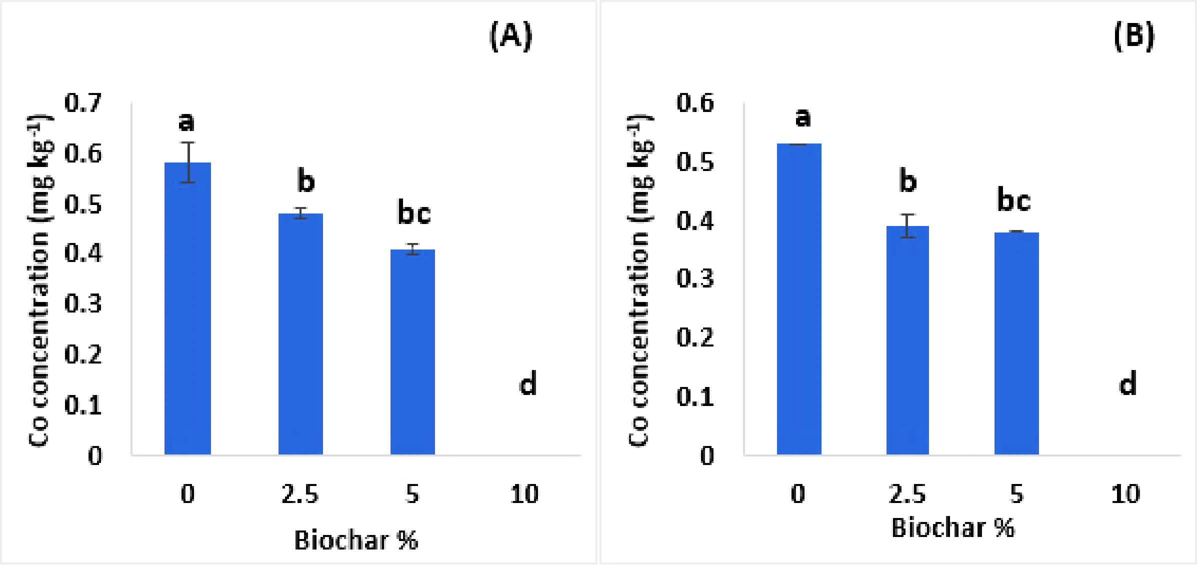


Figure 2.20: Co concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above-ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

The Co concentration in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants **(Figure 2.20).** The Co accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 0.58, 0.0.48, 0.41, and 0.00 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 0.53, 0.39, 0.38, and 0.00 mg kg^{-1}, respectively, in *Phleum pratense* L.

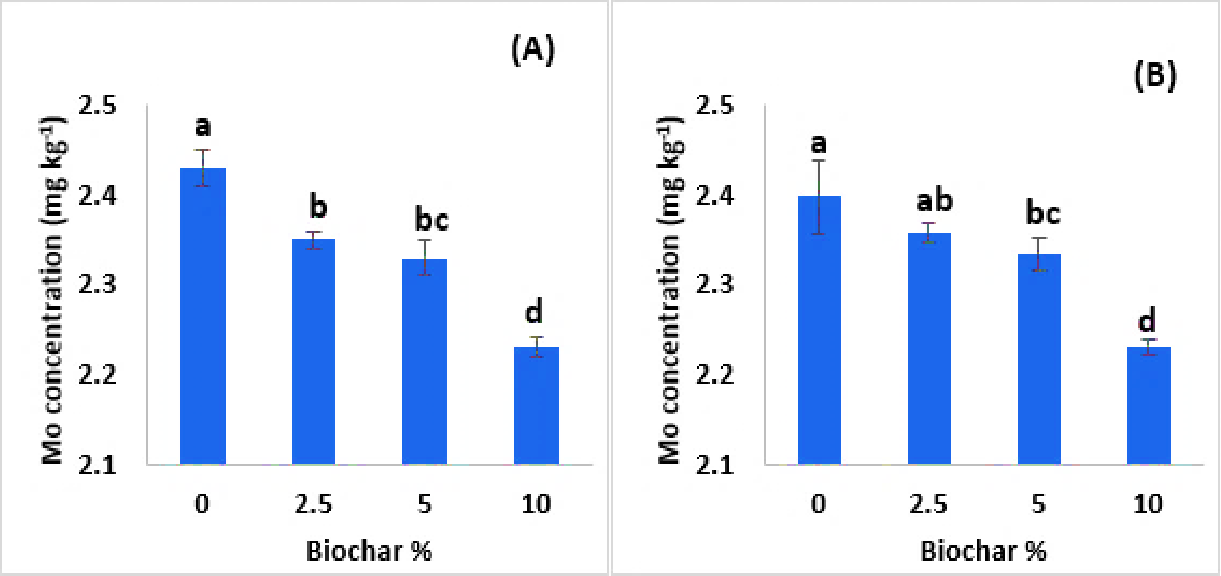


Figure 2.21: Mo concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above-ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Mo does not always get the attention it deserves. This essential element plays a very important role in the growth and development of plants (Reddy et al., 1997). In many agricultural soils, the content of molybdenum is somewhere in the range of 0.6 – 3.5 mg kg^{-1} ppm with an average of 2 mg kg^{-1}. In agricultural soils, Mo is present as many different complexes depending on the chemical speciation of the soil zone (Kaiser et al., 2005).

Plants typically take up molybdenum in the form of molybdate (Kaiser et al., 2005). One major factor controlling the amount of Mo that is available for the plants is the pH of the substrate (Reddy et al., 1997). The adsorption rate at neutrality is extremely low. The solubility increases as soils become acidic, lower pH. Molybdenum is essential for several functions, including the enzymes nitrate reductase and nitrogenase (enzymes that reduce nitrogen to usable forms) (Kaiser et al., 2005). Of all crops, legumes are those that require the highest amount of molybdenum.

Dissolved molybdenum available to plants is commonly found in the soluble MoO_4^{2-}anion form (Lindsay, 1979).

The Mo accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The Mo accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 2.43, 2.35, 2.33, and 2.23 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 2.40, 2.36, 2.33, and 2.23 mg kg^{-1}, respectively, in *Phleum pratense* L **(Figure 2.21).**

In *Phleum pratense* L treated with 2.5% biochar showed numerically lower Mo accumulation compared with control, but the different did not reach a significant level. however, Mo accumulation was significantly ($p < 0.05$) reduced in 5% and 10% biochar treatments compared with control.

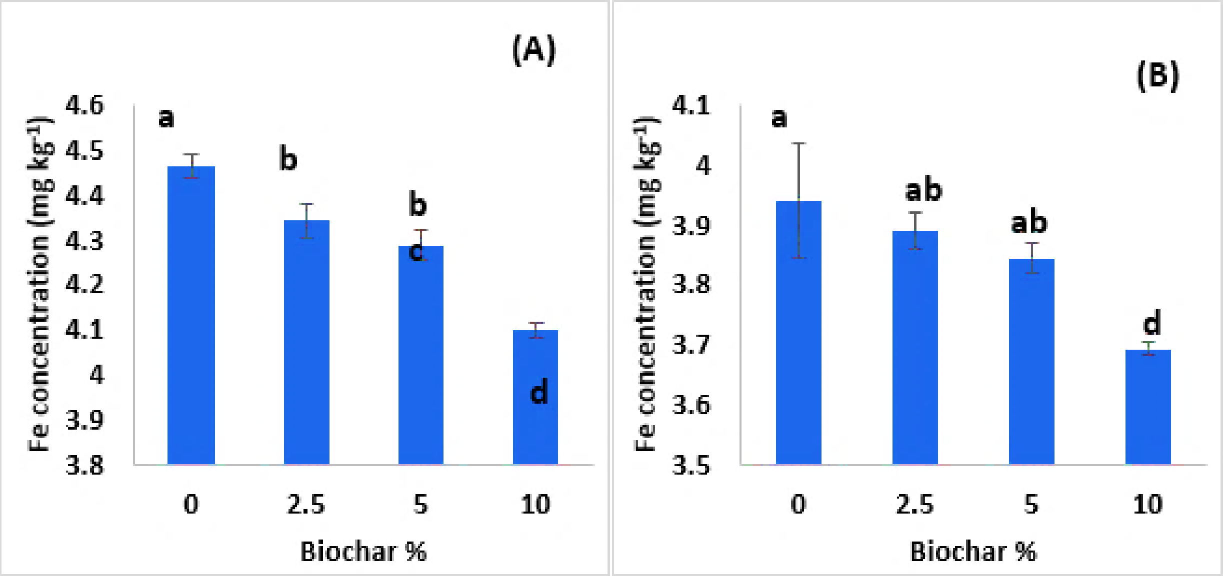


Figure 2.22: Fe concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above-ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Fe is essential for plant growth. Plants mainly acquire Fe from the rhizosphere. Although Fe is one of the most abundant metals in the earth's crust, its availability to plant roots is very low. The solubility of Fe in soils is largely controlled by Fe oxides; ferrihydrite, amorphous ferric hydroxide (Lindsay, 1991) Fe availability is dictated by the soil redox potential and pH (Morrissey & Guerinot, 2009). In soils that are aerobic or of higher pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides. At lower pH, the ferric Fe is freed from the oxide, and becomes more available for uptake by roots. Because 30% of the world's cropland is too alkaline for optimal plant growth (Marschner, 1995), and some staple crops, like rice, are especially susceptible to Fe deficiency (Takahashi et al., 2001) much research has focused on how plants cope with Fe limitation (Morrissey & Guerinot, 2009).

The Fe accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The Fe accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 4.46, 4.34, 4.28, and 4.09 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 3.94, 3.89, 3.85, and 3.69 mg kg^{-1}, respectively, in *Phleum pratense* L **(Figure 2.22).**

In *Phleum pratense* L treated with 2.5% biochar showed numerically lower Fe accumulation compared with control, but the different did not reach a significant level. However, Fe accumulation was significantly ($p < 0.05$) reduced 5% and 10% biochar treatment compared with control.

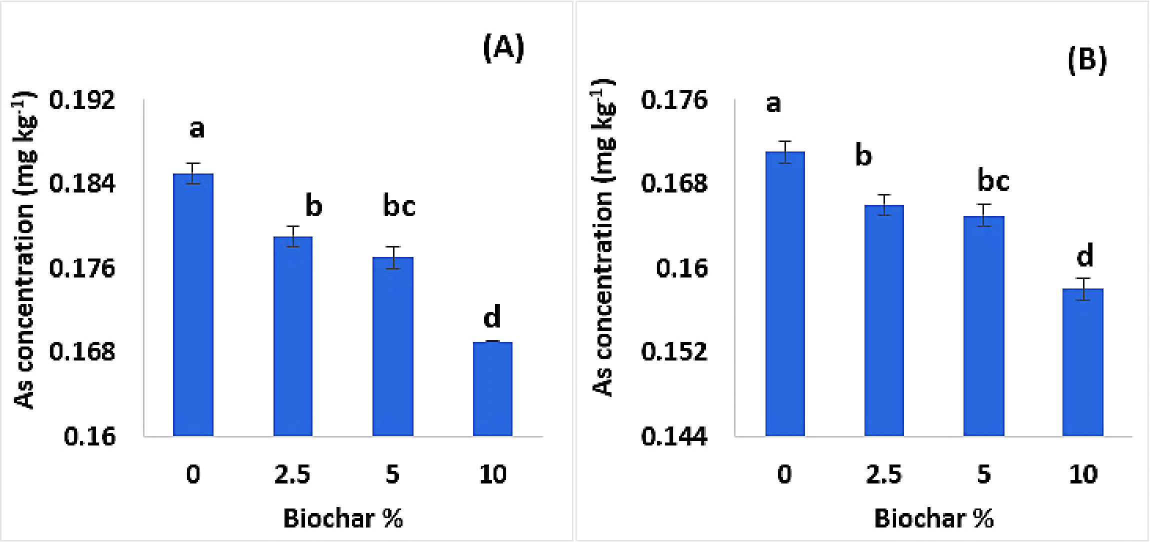


Figure 2.23: As concentration in (A) *Trifolium pratense* (B) *Phleum pratense* L (above-ground biomass) under different % biochar treatments. Mean values are presented with standard errors. Means values with same letter are not significantly ($p < 0.05$) different from each other

Environmental contamination with As is a global environmental, agricultural and health issue due to the highly toxic and carcinogenic nature of As (Abbas et al., 2018). Exposure of plants to As, even at very low concentration, can cause many morphological, physiological, and biochemical changes. The recent research on As in the soil-plant system indicates that As toxicity to plants varies with its speciation in plants, with the type of plant species, and with other soil factors controlling As accumulation in plants. Various plant species have different mechanisms of uptake, toxicity, and detoxification (Abbas et al., 2018).

Different soils have varying background concentrations of As depending on the parent material of soils; in most cases, the baseline soil As content can range from 5 to 10 mg kg^{-1} (Basu et al., 2014; Abbas et al., 2018). For European topsoil, an average As concentration of 7 mg kg^{-1} has been reported (Karczewska et al., 2007; Stafilov et al., 2010). On the other hand, peats and bog soils are relatively more enriched with As, where average soil As concentrations of up to 13 mg kg^{-1} have been noted (Rasheed et al., 2017; Abbas et al., 2018).

The As accumulation in plant biomass significantly ($p < 0.05$) reduced with biochar in both plants. The As accumulation in the 0, 2.5, 5, and 10% biochar amended soils were 0.185, 0.179, 0.177, and 0.169 mg kg^{-1}, respectively, in *Trifolium pratense*, whereas 0.171, 0.166, 0.165, and 0.158 mg kg^{-1}, respectively, in *Phleum pratense* L (**Figure 2.23**).

In *Phleum pratense* L treated with 2.5% biochar showed numerically lower As accumulation compared with control, but the different did not reach a significant level. However, As accumulation was significantly ($p < 0.05$) reduced in 5% and 10% biochar treatments compared with control.

It is well known that concentrations of Cd in edible vegetables range from 0.05 to 0.9 µg g^{-1} (DW) and leafy plants such as lettuce, cabbage, spinach contain relatively higher Cd than grain or fruit plants such as apple, barley, corn, oat and rice (Alloway,1995). Although Cd concentrations in plants grown on uncontaminated or unmineralized soils generally do not exceed 1.0 µg g^{-1} (DW) (Adriano, 1986; Alloway,1995), over 1 µg g^{-1} (DW) has been found in some plant leaves grown on contaminated soils from mining activities (Jung & Thornton, 1997).

Concentrations of Cd and Hg in both plants in all treatments were below the detection limit, which is less than 0.05 (µg kg ^{1}). It may due to the low-level concentration in studied soil.

2.10 Conclusion

This study was designed to investigate and control both leaching and bioavailability of heavy metal in fly-ash amended soil by adding different level of biochar as amendments. The addition of biochar (2.5, 5 and 10%) increased the soil pH significantly.

Each column went through a total of 11 consecutive leaching events (6.5 pore volume equivalent) which corresponds to one year's average precipitation and showed that the soil is not subjected to subsequent acidification. The leaching column experiment showed that the application of biochar to fly-ash amended soil can help reduce the leachability of Cu, Co, Ni, Cr, As, V, Zn, Fe and Pb significantly. The leachability of metals decreases when the soil is amended with 2.5, 5 or 10% of biochar. The accumulated mass of each metal in the leachate varied depending on the treatment applied. In the original fly-ash treated soil the amount of metals leached was the highest in the absence of biochar except for Mo. The total amount of heavy metal leached from all treatments is very low compared to the total available heavy metal.

The pot experiment results showed a significant increased biomass production in plants studied (*Trifolium pratense* and *Phleum pratense* L) when compared to the control. The addition of biochar reduces the bioavailability and plant uptake of Cu, Co, Ni, Cr, V, As, Fe, Mo, Zn and Pb. Mean concentrations of Cd and Hg, in both plants in all treatments were below the detection limit. It may be due to the low-level concentration in studied soil.

The observed immobilization could be essentially through the rise of soil pH, with an increase of metal retention on soil particles. The application of biochar that can immobilize heavy metals could provide a cost-effective and sustainable solution for the heavy metal stabilization of fly-ash added soil. According to comparison of the effectiveness of different treatments 10% biochar amended soil was recognized as one of the best treatment in reducing both leaching and bioavailability of metals with no observed decline in plant biomass

2.11 Recommendation

However, the estimation of the leaching potential and the bioavailability of toxic elements in the field is important in assessing the possible environmental impact associated with the utilization of fly-ash along with biochar addition in crop field. To have a complete picture about biochar role in heavy metal immobilization and bioavailability, further experiments should include long term biochar field application on different soil types, biochar rates, and types and dose of metal contamination. Furthermore, more research must be carried out for assessing whether immobilization of contaminants in soil is long enough irreversible to remain stable under natural condition.

2.12 References

Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N. K., Khan, M. I., … Natasha. (2018). Arsenic uptake, toxicity, detoxification, and speciation in plants: Physiological, biochemical, and molecular aspects. International Journal of Environmental Research and Public Health, 15(1). https://doi.org/10.3390/ijerph15010059

Abujabhah, I. S., Doyle, R., Bound, S. A., & Bowman, J. P. (2016). The effect of biochar loading rates on soil fertility, soil biomass, potential nitrification, and soil community metabolic profiles in three different soils. Journal of Soils and Sediments, 16(9), 2211–2222. https://doi.org/10.1007/s11368-016-1411-8

Adriano, D.C. Trace elements in the terrestrial Environment. Springer-Verlag Inc.: New York, 1986; pp. 1- 45.

Ahmad, M., Lee, s. s., Lim, J. E., Lee, S. E., Cho, J. S., Moon, D. H., Hashimoto, Y., Ok, Y. S. 2014. Speciation and phyto availability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and biochar: EXAFS spectroscopy and chemical extractions. Chemosphere 95, 433–441

Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., … Ok, Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: A review. Chemosphere, 99, 19–33. https://doi.org/10.1016/j.chemosphere.2013.10.071

Ahmad, S., Ghafoor, A., Akhtar, M. E., and Khan, M. Z. 2013. Ionic displacement and reclamation of salinesodic soils using chemical amendments and crop rotation, Land Degrad. Develop., 24, 170–178

Ahmedna, M., Marshall, W., E., Husseiny A., A., Rao R., M., Goktepe, I. (2004). The use of nutshell carbons in drinking water filters for removal of trace metals. Water Res. 38, 1062- 1068

Alloway, B.J. Heavy Metals in Soils. Blackie Academic and Professional: London, 1995; pp. 7 - 39.

Almaroai, Y.A., Usman, A.R.A., Ahmad, M., Moon, D.H., Cho, J.S., Joo, Y.K., Jeon, C., Lee, S.S., Ok, Y.S., 2013. Effects of biochar, cow bone, and eggshell on Pb availability to maize in contaminated soil irrigated with saline water. Environ. Earth Sci.. http://dx.doi.org/10.1007/s12665-013-2533-6.

Al-Wabel, M., I., Usman, A., R, .A., El-Naggar A., H., Aly A., A., Ibrahim H., M., Elmaghraby S., Al-Omran. 2015. Conocarpusbiochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi Journal of Biological Sciences 22, 503–511

Angin, D., Sensöz, S. 2014. Effect of pyrolysis temperature on chemical and surface properties of biochar of rapeseed (Brassica napus L.). Int. J. Phytoremediat., 16, 684-693

Atkinson, C.J.; Fitzgerald, J.D.; Hipps, N.A. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil **2010**, 337, 1–18.

Bashir, S., Hussain, Q. , Akmal, M., Riaz, M., Hu, H. , Ijaz, S. S., Iqbal, M., Abro S., Mehmood, S., Ahmad, M. 2017, Sugarcane bagasse-derived biochar reduces the cadmium and 49 chromium bioavailability to mash bean and enhances the microbial activity in contaminated soil. Journal of Soils and Sediments, 1-13.

Basu A., Saha D., Saha R., Ghosh T., Saha B. A review on sources, toxicity and remediation technologies for removing arsenic from drinking water. Res. Chem. Intermed. 2014;40:447–485.

Batish, D., & Kohli, R. (2013). Chromium Toxicity and Tolerance in Plants. (September). https://doi.org/10.1007/s10311-013-0407-5

Beesley L, Marmiroli M (2011) The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. Environ Pollut 159(2):474–480

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., & Sizmur, T. (2011). A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. Environmental Pollution, 159(12), 3269–3282. https://doi.org/10.1016/j.envpol.2011.07.023

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158, 2282e2287

Bian.J., S. Joseph, L.Q. Cui, G.X. Pan, L.Q.Li, X.Y. Liu, A. Zhang, H. Rutlidge, S.W. Wong, C. Ch ia, C. Marjo, B. Gong, P. Munroe, S.Donne. 2014. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. J. Hazard. Mater. 272. 121-128

Bolan, N., Kunhikrishnanc, A., Thangarajan, R., J., Kumpiene, Parke, J., Makinof, T., Kirkhamg, M. B., Scheckel, K. 2014. Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize? Journal of Hazardous Materials 266, 141–166

Bolan, N.S., Adriano, D.C., Curtin, D., 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. Advances in Agronomy 78, 215e272.

Bolan, N.S., Duraisamy, P., Mani, S.: Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. Aust. J. Soil Res. 41(1), 533–555 (2003)

Bradbury MH, Baeyens B. Sorption modelling on illite Part I: Titration measurements and the sorption of Ni, Co, Eu and Sn. Geochim. Cosmochim.Acta. 2009;73:990–10dd03. doi: 10.1016/j.gca.2008.11.017

Camberato, J. J., Gagnon, B., Angers, D. A., Chantigny, M. H., & Pan, W. L. (2011). Pulp and paper mill by-products as soil amendments and plant nutrient sources. Canadian Journal of Soil Science, 86(4), 641–653. https://doi.org/10.4141/s05-120

Campbell, A.G. 1990 Recycling and disposing of wood ash. Tappi J. 73(9) 141-146

Cao X, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45(11):4884–4889

Cao, X., Ma, L., Gao, B., Harris, W. 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ. Sci. Technol. 43(9), 3285-3291.

Cappuyns V., R. Swennen, 2008. The application of pHstat leaching tests to assess the pH dependent release of trace metals from soils, sediments and waste materials. Journal of Hazardous Materials 158, 185-195

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 Technology, 102(19), 8877–8884.

Choppala, G.K., Bolan, N.S., Mallavarapu, M., Chen, Z., Naidu, R., 2012. The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. J. Environ. Qual. 41, 1–10

Churchill, D. and Kirby, A.: CB Pulp and Paper Ash Diversion, Phase 1: Initial Ash Testing, Project No 09-14., 2010

Clemente J.S., Beauchemin S., MacKinnon T., Martin J., Johnston C.T., Joern B. 2017. .Initial biochar properties related to the removal of As, Se, Pb, Cd, Cu, Ni, and Zn from an acidic suspension. Chemosphere 170, 216–224.

Cui, Y.-J., Zhu, Y.-G., Zhai, R.-H., Chen, D.-Y., Huang, Y.-Z., Qiu, Y., Liang, J.-Z., 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China.Environ. Int. 30, 785–791

Demeyer, A., Voundi Nkana, J. C., & Verloo, M. G. (2001). Characteristics of wood ash and influence on soil properties and nutrient uptake: An overview. Bioresource Technology, 77(3), 287–295. https://doi.org/10.1016/S0960-8524(00)00043-2

Dong D, Feng O, McGrouther K, Yang M, Wang H, Wu W. Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. J Soils Sediments. 2015;15: 153–162.

Downie, A., Crosky, A., Munroe, P. (2009). Physical properties of biochar. In: Biochar for Environmental Management: Science and Technology (Eds. Lehmann, J. & Joseph, S.), Earthscan

Dube BK, Tewari K, Chatterjee J, Chatterjee C (2003) Excess chromium alters uptake and translocation of certain nutrients in Citrullus. Chemosphere 53:1147–1153

Fahmi, A.H.; Samsuri, A.W.; Jol, H.; Singh, D. (2018). Bioavailability and leaching of Cd and Pb from contaminated soil amended with different sizes of biochar. R. Soc. Open Sci. 2018, 5, 181328

Fellet, G., Marchiol, L., Delle Vedove, G., Peressotti, A., 2011. Application of biochar on mine tailings: effects and perspectives for land reclamation. Chemosphere 83, 1262e1297

Fergusson, J.E. The Heavy Elements: Chemistry, Environmental Impacts and Health Effects. Pergamon Press: Oxford, 1990; pp. 377 – 405.

Głąb T, Palmowska J, Zaleski T, Gondek K. Effect of biochar application on soil hydrological properties and physical quality of sandy soil. Geoderma. 2016;281: 11–20.

Glaser B, Parr M, Braun C, Kopolo G (2009) Biochar is carbon negative. Nat Geosci 2(1):2

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal: a review. Biology and Fertility of Soils 35: 219-230

Godlewska P., Schmidt H.P., Ok Y.S. (2017). Biochar for composting improvement and contaminants reduction. A review. Bioresource. Technology, In press, Available online 18 July 2017

Hagner M., Kemppainen R., Jauhiainen L., Tiilikkala K., Setälä H. 2016. The effects of birch (Betula spp.) biochar and pyrolysis temperature on soil properties and plant growth. Soil and Tillage Research. 163, 224-234

Hartley, W., Dickinson, N.M., Riby, P., Lepp, N.W. (2009). Arsenic mobility in brownfield soils amended with greenwaste compost or biochar and planted with Miscanthus. Environmental Pollution 157, 2654e2662

Houben, D., Evrard, L., & Sonnet, P. (2013). Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. Chemosphere, 92(11), 1450–1457. https://doi.org/10.1016/j.chemosphere.2013.03.055

Huffman EW Jr, Allaway WH (1973) Chromium in plants: distribu-tion in tissues, organelles and extracts, and availability of beanleaf Cr to animals. J Agric Food Chem 21:982–986

Hussain M., M. Farooq, A. Nawaz, A.M. Al-Sadi, Z.M. Solaiman, S.S. Alghamdi, U. Ammara, Y.S. Ok, K.H.M. Siddique. (2016). Biochar for crop production: potential benefits and risks. J. Soils Sediments, 1-32

Ibrahim, H.M., Al-Wabel, M.I., Usman, A.R.A., Al-Omran, A. (2013) .Effect of Conocarpus biochar application on the hydraulic properties of a sandy loam soil. Soil Sci. 178,165–173.

Igalavithana A.D., J. Park, Ch. Ryu, Y. H. Lee, S. S. Lee. 2017. Slow pyrolyzed biochars from crop residues for soil metal(loid) immobilization and microbial community abundance in contaminated agricultural soils. Chemosphere 177, 157-166

Ippolito, J.A., J.M. Novak, D.G. Strawn, K.G. Scheckel, M. Ahmedna, and M.A.S. Niandou. (2012). Macroscopic and molecular approaches of copper sorption by a steam activated biochar. J. Environ. Qual. 41:1150–1156. doi:10.2134/jeq2011.0113

Jung, M.C., Thornton, I., (1997). Environmental contamination and seasonal variation of metals in soils, plants and waters in the paddy fields around a Pb-Zn mine in Korea.Sci.Tot. Environ.198, 105–121.

Kaiser, B. N., Gridley, K. L., Brady, J. N., Phillips, T., & Tyerman, S. D. (2005). The role of molybdenum in agricultural plant production. Annals of Botany, 96(5), 745–754. https://doi.org/10.1093/aob/mci226

Karami, N., Clemente, R., Moreno-Jiménez, E., Lepp, N., Beesley, L. (2011). Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass (Lolium perenne). Journal of Hazardous Materials 191, 41e48

Karczewska A., Bogda A., Krysiak A.(2007) Arsenic in soils in the areas of former mining and mineral processing in Lower Silesia, southwestern Poland. Trace Met. Contam. Environ. 2007;9:411–440

Keiluweit M, et al. Dynamic molecular structure of plant biomass-derived black carbon (biochar) Environ. Sci. Technol. 2010;44:1247–1253. doi: 10.1021/es9031419

Kishor, P., K, G. A., & Dileep Kumar. (2010). Use of Flyash in Agriculture: A Way to Improve Soil Fertility and its Productivity. Asian Journal of Agricultural Research, 4, 1–14.

Komonweeraket, K., Cetin, B., Aydilek, A. H., Benson, C. H., & Edil, T. B. (2015). Effects of pH on the leaching mechanisms of elements from fly ash mixed soils Effects of pH on the leaching mechanisms of elements from fly ash mixed soils. FUEL, 140(December 2018), 788–802. https://doi.org/10.1016/j.fuel.2014.09.068

Koyama S, Katagiri T, Minamikawa K, Kato M, Hayashi H. (2016) Effects of rice husk charcoal application on rice yield, methane emission, and soil carbon sequestration in Andosol paddy soil. JARQ.;50 (4): 319–327.

Kumpiene, J. (2010). Trace element immobilization in soil using amendments. In P. Hooda (Ed.), Trace elements in soils (pp. 353–380). Wiltshire: Wiley

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219.

Lehmann J., Czimczik C., Laird D., Sohi S. (2009). Stability of biochar in soil. P. 183-198. Biochar for environmental management. Science and technology. Edited by Lehmann J. and Joseph S. 416p. 57.

Lehmann J., Joseph S. (2009). Biochar for Environmental Management: An Introduction. P. 1- 9. Biochar for environmental management. Science and technology. Edited by Lehmann J. and Joseph S. 416p.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., & Crowle, D. (2011). Biochar effects on soil biota- A review. Soil Biology and Biochemistry, 43, 1812-1836

Li H., X. Dong, E.B. da Silva, L. M. de Oliveira, Y. Chen, Lena Q. Ma. (2017). Mechanisms of metal sorption by biochars: Biochar characteristics and modifications. Chemosphere 178, 466–478.

Li H., Ye X., Geng Zh., Zhou H., Guo X., Zhang Y., Zhao H., Wang G. (2016). The influence of biochar type on long-term stabilization for Cd and Cu in contaminated paddy soils. Journal of Hazardous Materials 304, 40–48

Lickacz, J. (2002). Wood Ash - An Alternative Liming Material for Agricultural Soils. (February), 1–6.

Lu K., Yang X., Shen J., Robinson B., Huang H., Liu D., Bolan N., Pei J., Wang H. 2014. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to Sedum plumbizincicola. Agriculture, Ecosystems and Environment 191, 124–132

Major, J., M. Rondon, D. Molina, S. J. Riha, and J. Lehmann. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant and Soil 333:117–128. DOI 10.1007/s11104-010-0327-0

Marschner H. Mineral Nutrition of Higher Plants. 2nd ed. Boston: Academic Press; 1995

Martin, H.W. & Kaplan, D.I. (1998). Temporal changes in cadmium, thallium, and vanadium mobility in soil and phytoavailability under field conditions. Water Air and Soil Pollution, 101(1-4), pp. 399-410

McLaughlin H., Anderson P.S., Shields F.E., Reed T.B., 2009. All biochars are not created equal and how to tell them apart. Presented at: North Americal Biochars Conference, 9–12 August, Boulder, CO., USA.

Mikkonen, A. & Tummavuori, J. (1994b). Retention of vanadium (V) by three Finnish mineral soils. European Journal of Soil Science, 45(3), pp. 361- 368.

Mohan, D., Pittman Jr., C.U., 2007. Arsenic removal from water/wastewater using environment e a critical review. Journal of Hazardous Materials 142, 1e53.

Morrissey, J., & Guerinot, M. Lou. (2009). Iron uptake and transport in plants: The good, the bad, and the ionome. Chemical Reviews, 109(10), 4553–4567. https://doi.org/10.1021/cr900112r

Naylor, L. M., & Schmidt, E. (1989). Paper mill wood ash as a fertilizer and liming material: field trials. Tappi Journal, 72(6), 199–206.

Neiboer E, Richardson DHS (1980) The replacement of the nonde-script term heavy metals by a biologically and chemicallysignificant classification of metal ions. Environ Pollut Ser BChem Phys 1:3–26

Novak J.M., Lima I., Xing B., Gaskin J.W., Steiner C., Das K.C., Ahmedna M., Rehrah D., Watts D.W., Busscher W.J. & Schomberg H. (2009). Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. Annals of Environmental Science 3: 195-206

Park, J.H., Choppala, G.K., Bolan, N.S., Chung, J.W., Cuasavathi, T. 2011. Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant Soil 348, 439–451

Peacock CL, Sherman DM (2004) Copper(II) sorption onto goethite, hematite and lepidocrocite: a surface complexation model based on ab initio molecular geometries and EXAFS spectroscopy. Geochim Cosmochim Acta 68(12):2623–2637

Pitman, R. M. (2006). Wood ash use in forestry - A review of the environmental impacts. Forestry, 79(5), 563–588. https://doi.org/10.1093/forestry/cpl041

Pöykiö, R., Nurmesniemi, H., Perämäki, P., Kuokkanen, T., & Välimäki, I. (2005). Leachability of metals in fly ash from a pulp and paper mill complex and environmental risk characterisation for eco-efficient utilization of the fly ash as a fertilizer. Chemical Speciation and Bioavailability, 17(1), 1–9. https://doi.org/10.3184/095422905782774964

Puga, A. P., Melo, L. C. A., de Abreu, C. A., Coscione, A. R., & Paz-Ferreiro, J. (2016). Leaching and fractionation of heavy metals in mining soils amended with biochar. Soil and Tillage Research, 164, 25–33. https://doi.org/10.1016/j.still.2016.01.008

Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol Fertil Soils. 2012;48: 271–284

Rao, E. Anthony, L. Jia and A. Macchi, "Carbonation of FBC ash by sonochemical treatment," Fuel, vol. 86, no. 16, pp. 2603-2615, 2007

Rasheed H, Kay P, Slack R, Gong YY, Carter A Sci Total Environ. 2017 Apr 15; 584-585():631-641.

Reddy KJ, Munn LC, Wang L. 1997. Chemistry and mineralogy of molybdenum in soils. In: Gupta UC, ed. Molybdenum in agriculture. Cambridge: Cambridge University Press.

Richter RO, Theis TL (1980) Nickel speciation in a soil/water system. In: Nriagu JO (ed) Nickel in the Environment

Shanker AK, Djanaguiraman M, Venkateswarlu B (2009) Chromiuminteractions in plants: current status and future strategies.Metallomics 1:375–383

Sharifi, M., Cheema, M., McVicar, K., LeBlanc, L., & Fillmore, S. (2013). Evaluation of liming properties and potassium bioavailability of three Atlantic Canada wood ash sources. Canadian Journal of Plant Science, 93(6), 1209–1216. https://doi.org/10.4141/cjps2013-168

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. Indian Journal of Pharmacology, 43(3), 246–253. http://doi.org/10.4103/0253-7613.81505

Sohi, S. P. (2012). Carbon storage with benefits. Science 338, 1034–1035

Sohi, S., Lopez-Capel, E., Krull, E. & Bol, R. (2009). Biochar, climate change and soil: A review to guide future research. CSIRO Land and Water Science Report 05/09, 64pp

Steiner, C., Teixeira, W., Lehmann, J., Nehls, T., de Macêdo, J., Blum, W., and 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 and Soil 291(1): 275- 290

Takahashi MT, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. Nat. Biotechnol. 2001;19:466

Tang J., W. Zhu, R. Kookana, A.Katayama. 2013. Characteristics of biochar and its application in remediation of contaminated soil. Journal of Bioscience and Bioengineering 116, 653–659

Uchimiya M, Wartelle LH, Klasson KT, Fortier CA, Lima IM. 2011 Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. J. Agric. Food Chem. 59, 2501–2510. (doi:10.1021/jf104206c)

Uchimiya, M., Bannon, D.I., Wartelle, L.H., Lima, I.M., Klasson, K.T., 2012. Lead retention by broiler litter biochars in small arms range soil: impact of pyrolysis temperature. J. Agric. Food Chem. 60, 5035–5044

Uchimiya, M., I.M. Lima, K.T. Klasson, and L.H. Wartelle. 2010. Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. Chemosphere 80:935–940. doi:10.1016/j.chemosphere.2010.05.020

Uchimiya, M., K.T. Klasson, L.H. Wartelle, and I.M. Lima. 2011b. Influence of soil properties on heavy metal sequestration by biochar amendment: 2. Copper desorption isotherms. Chemosphere 82:1438–1447. doi:10.1016/j.chemosphere.2010.11.078

Uchimiya, M., L.H. Wartelle, K.T. Klasson, C.A. Fortier, and I.M. Lima. 2011c. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. J. Agric. Food Chem. 59:2501– 2510. doi:10.1021/jf104206c

Uchimiya, M., Lima, I.M., Klasson, T., Wartelle, L.H., 2010. Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere 80, 935e940.

Verheijen, F., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2010. Biochar application to soils. A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. European Commission, Italy

Vithanage M., Herath I., Joseph S., Bundschuh J., Bolan N., Ok Y. S., Kirkham M.B., Rinklebe J. 2017. Interaction of arsenic with biochar in soil and water: A critical review. Carbon, Vol. 113, 219-230.

Williams, T.M., Hollis, CA, and Smith, B.R. 1996. Forest soil and water chemistry following bark boiler bottom ash applications. J. Environ. Qual. 25: 955-961

Win, K. T., Okazaki, K., Ookawa, T., Yokoyama, T., & Id, Y. O. (2019). Influence of rice-husk biochar and Bacillus pumilus strain TUAT-1 on yield , biomass production , and nutrient uptake in two forage rice genotypes. 1–21.

Woolf D., J.E. Amonette, F. A. Street-Perrott, J. Lehmann, S, Joseph. 2010. Sustainable biochar to mitigate global climate change. Nat Commun. 1

Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils : A Review of Sources , Chemistry , Risks and Best Available Strategies for Remediation. 2011. https://doi.org/10.5402/2011/402647

Xu G., Lv Y., Sun J., Shao H., Wei L. Review. 2012. Recent Advances in Biochar Applications in Agricultural Soils: Benefits and Environmental Implications. Clean – Soil, Air, Water, 1–6.

Xu X.Y., Cao X.D., Zhao L., Wang H., Yu H., Gao B. 2013. Removal of Cu, Zn, and Cd from aqueous solutions by the dairy manure-derived biochar. Environ. Sci. Pollut. Res. 20, 358-368

Yuan, J.H., Xu, R.K., 2011. The amelioration effects of low temperature biochar generated from nine crop residues on an acidic ultisol. Soil Use and Management 27, 110e115

Zhang, R. H., Li, Z. G., Liu, X. D., Wang, B. cai, Zhou, G. L., Huang, X. X., … Brooks, M. (2017). Immobilization and bioavailability of heavy metals in greenhouse soils amended with rice straw-derived biochar. Ecological Engineering, 98, 183–188. https://doi.org/10.1016/j.ecoleng.2016.10.057

Zhang, X., Wang, H., He, L., Lu, K., Sarmah, A., Li, J., … Huang, H. (2013). Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environmental Science and Pollution Research, 20(12), 8472–8483. https://doi.org/10.1007/s11356-013-1659-0

Zhou, L., Liu, Y., Liu, S., Yin, Y., Zeng, G., Tan, X., … Huang, X. (2016). Investigation of the adsorption-reduction mechanisms of hexavalent chromium by ramie biochars of different pyrolytic temperatures. Bioresource Technology, 218, 351–359. https://doi.org/10.1016/j.biortech.2016.06.102

Zimmerman, A.R., Gao, B., Ahn, M.Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 43, 1169–1179

Zweiten, L.V., Singh, B., Joseph, S., Kimber, S., Cowei, A., Chan, K.Y., 2009. Biochar and emissions of non-CO2 greenhouse gases from soil. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management Science and Technology. Earthscans, UK, pp. 227–250

Overall conclusions

This study was conducted to assess the potential of fly-ash from CBPPL as an alternate liming material for Newfoundland agricultural soil. Heavy metal concentration and liming effect of the fly-ash with soil were analysed and compared with soil and compost quality guidelines. Results showed that using fly-ash as a soil amendment and liming material can increase soil pH.

The metal concentrations values in the CBPPL fly-ash were lower than those in the CCME compost categories B guidelines, and therefore the fly-ash could potentially be used in the category B compost. The concentrations of Ni, Zn and Mo in fly-ash were slightly higher than those in the CCME compost A guidelines. The final product may pass as a category A compost when fly-ash applied with other soil amendments which are low in Ni, Zn and Mo concentrations

According to the CFIA standards, only part of the fly-ash requirement can be substituted by CBPPL fly-ash. The percentage may vary depending on soil initial pH and desired pH and which crop is going to be grown. This percentage can be increased when fly-ash applied with other soil amendments which are low in trace element concentrations. Also, maximum allowable ash can be applied annually to forest land and marginal land to improve their fertility level and productivity.

A leaching experiment and pot experiment were conducted in the laboratory condition to investigate and control both leaching and bioavailability of heavy metals in fly-ash amended soil through the addition of different % biochar as amendments. The addition of biochar (2.5, 5 and 10%) increased the soil pH significantly. Soil is not subjected to subsequent acidification after it went through a total of 11 consecutive leaching events which corresponds to one year’s average precipitation

The leaching column experiment showed that the application of biochar to fly-ash amended soil can help reduce the leachability of Cu, Co, Ni, Cr, As, V, Zn, Fe and Pb, notably because it raises the soil pH and the pH of leachate. Moreover, provided soil is not subjected to subsequent acidification, the leachability of metals decreases (except for Mo, which increased) when the soil is amended by 2.5, 5 or 10% of biochar. The cumulative mass of each metal in the leachate varied depending on the treatment applied. Total heavy metal leached from all treatments were very low when compared to the total available metal in each column. In the non-biochar amended soil, the cumulative amount of metals leached was very high compared to other treatments except for Mo. The maximum metal concentrations in the leachate were still below the Quality Standards for drinking water, thereby suggesting that leaching from the fly-ash amended soil in this study is unlikely to cause contamination to groundwater.

2.5, 5 and 10% biochar amended soil showed a significantly increased biomass production in plants (*Trifolium pratense* and *Phleum pratensec* L) studied when compared to the non biochar amended soil. The addition of biochar reduces the bioavailability Cu, Co, Ni, Cr, V, As, Fe, Mo, Zn and Pb. Concentrations of Cd and Hg in both plants in all treatments were below the detection limit. It may due to the low level and/or zero level concentration in studied fly-ash amended soil

The observed immobilization could be essentially through the rise of soil pH, with an increase of metal retention on soil particles. The application of biochar that can immobilize heavy metals could provide a cost-effective and sustainable solution for the heavy metal stabilization of fly-ash added soil. According to the comparison of the effectiveness of different treatments in terms of reducing both leaching and bioavailability of metals with no observed decline in plant biomass, 10% biochar amended soil was recognized as one of the best compromises.

Recommendation

CBPPL is planning to purchase a bark dryer which will allow them to reduce oil consumption, and it should improve the quality of the ash with respect to metal contents. The fly-ash will need to be tested after the bark dryer is put in use. Continuous fly-ash sampling and analysis will be required throughout the year to monitor the temporal variability of fly-ash quality. Specific guidelines should be developed for fly-ash applications on forest and/or agricultural soils in the province of NL. However, the estimation of the leaching potential and the bioavailability of toxic elements in the field level is important in assessing the possible environmental impact associated with the utilization of ash along with biochar addition.

To have a complete picture about biochar role in heavy metal mobilization and bioavailability, further experiments should include long term biochar field application on different kinds of soils, levels, and types/ dose of metal contamination. Furthermore, more research must be done for assessing whether immobilization of contaminants in soil is long term and/or irreversible to remain stable under natural conditions.

The focus of future work should determine the repeatability of our results on a variety of contaminated soils and the mechanisms of individual element retention by a variety of biochars in the light of dynamic soil physical, microbial and chemical conditions found in the field.

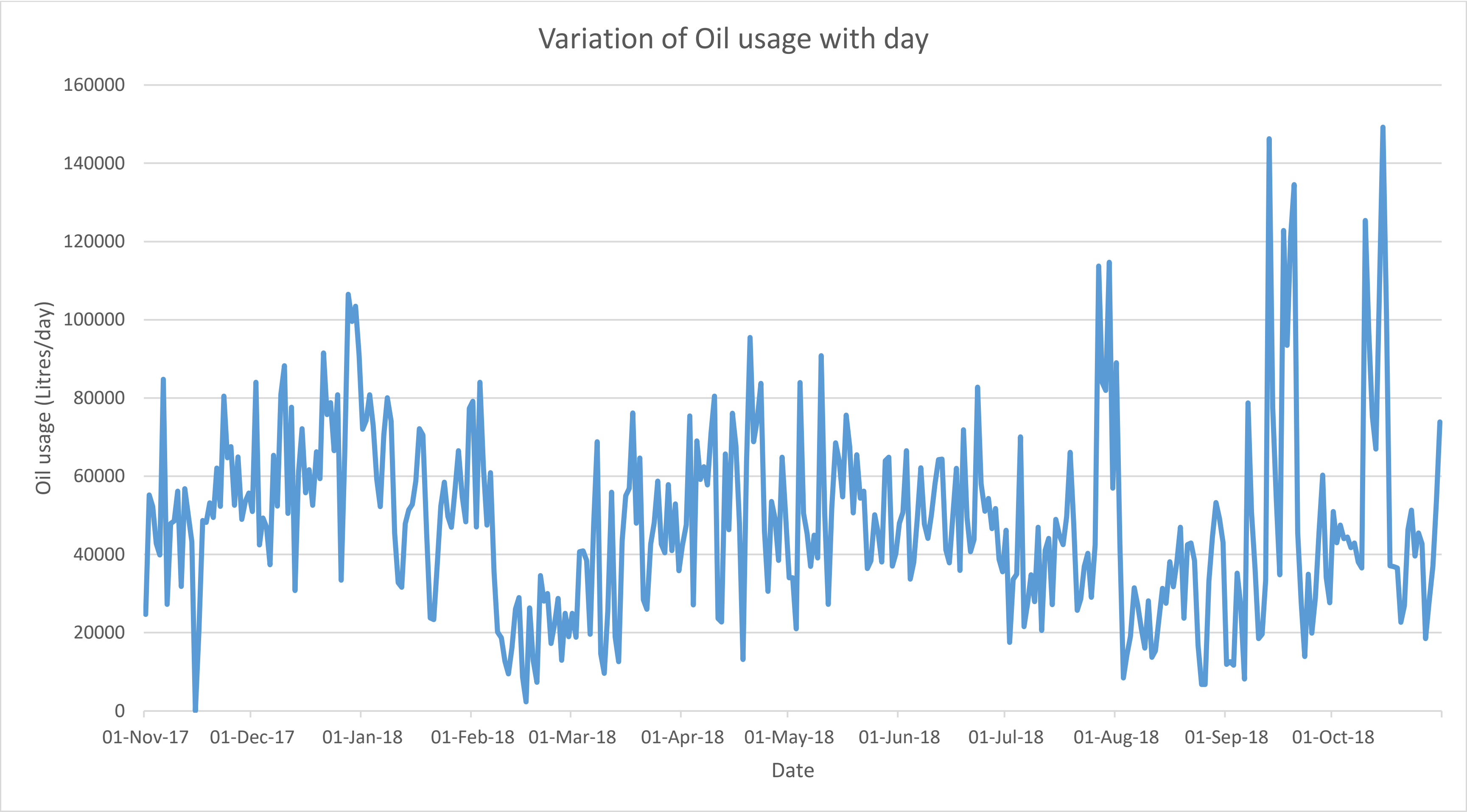
Hence, future studies are needed to determine the long-term effect of biochar for different crop biomass production in different soils at field and regional scales. Furthermore, it is important to evaluate the soil and plant response of biochar produced from different feedstocks and production temperatures which are available locally to ensure the cost-efficiency.

Appendices

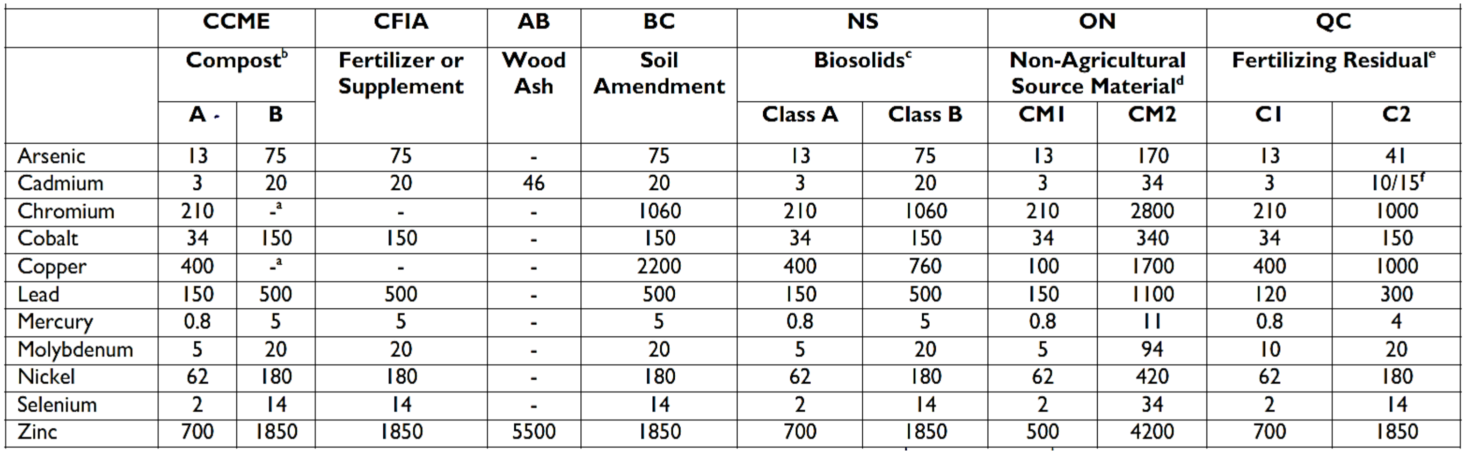
Appendix 1. CBPPL ash composite sample collection data

Date	Time	Steam from Bark	Steam from Oil	total	%biomass	%oil
5-Oct	14:05	156000	9000	165000	94.5	5.5
10-Oct	9:30	147000	29000	176000	83.5	16.5
11-Oct	9:30	171000	0	171000	100.0	0.0
12-Oct	9:35	123000	23000	146000	84.2	15.8
13-Oct	10:30	145000	0	145000	100.0	0.0
14-Oct	14:00	143000	0	143000	100.0	0.0
15-Oct	10:45	130000	45000	175000	74.3	25.7
16-Oct	9:45	148000	0	148000	100.0	0.0
17-Oct	11:00	138000	0	138000	100.0	0.0
18-Oct	10:45	131000	34000	165000	79.4	20.6
19-Oct	9:30	109000	53000	162000	67.3	32.7
21-Oct	11:20	144000	16500	160500	89.7	10.3
23-Oct	11:30	122000	23000	145000	84.1	15.9
24-Oct	11:10	133000	27000	160000	83.1	16.9
25-Oct	10:45	130000	30000	160000	81.3	18.8

Appendix 2. Variation of oil usage with day



Appendix 3. Variability in the trace metal limits (mg kg-1 dry weight) applied to wood ash used as a soil amendment across Canada **(Hannam et al., 2016)**



	CCME		CFIA	AB	BC	NS		ON		QC	
	Compost[b]		Fertilizer or Supplement	Wood Ash	Soil Amendment	Biosolids[c]		Non-Agricultural Source Material[d]		Fertilizing Residual[e]	
	A	B				Class A	Class B	CM1	CM2	C1	C2
Arsenic	13	75	75	-	75	13	75	13	170	13	41
Cadmium	3	20	20	46	20	3	20	3	34	3	10/15[f]
Chromium	210	-[a]	-	-	1060	210	1060	210	2800	210	1000
Cobalt	34	150	150	-	150	34	150	34	340	34	150
Copper	400	-[a]	-	-	2200	400	760	100	1700	400	1000
Lead	150	500	500	-	500	150	500	150	1100	120	300
Mercury	0.8	5	5	-	5	0.8	5	0.8	11	0.8	4
Molybdenum	5	20	20	-	20	5	20	5	94	10	20
Nickel	62	180	180	-	180	62	180	62	420	62	180
Selenium	2	14	14	-	14	2	14	2	34	2	14
Zinc	700	1850	1850	5500	1850	700	1850	500	4200	700	1850

- Trace metal limits for chromium and copper have not been established but would be 1060 mg kg-1 and 757 mg kg-1, respectively, if calculated with the same method used to establish limits for the other nine trace element(CCME, 2005).
- **Category A Compost**: wood ash with ‘trace element’ concentrations that fall below the limits set for category A compost by the Canadian Council of Ministers of the Environment (2005) is considered ‘unrestricted’ and can be used as a soil amendment in any application(CCME, 2005);
- **Category B Compost:** wood ash with ‘trace element’ concentrations that fall above the limits for category A compost but below the limits for category B compost has ‘restricted’ use and ‘may require additional control when deemed necessary by a province or territory’; wood ash with ‘trace element’ concentrations above the limits for Category B Compost ‘must be used or disposed of appropriately’(CCME, 2005).
- **Class A Biosolids:** wood ash with trace metal concentrations that fall below the ‘maximum acceptable metal concentrations’ for Class A Biosolids can get approval from Nova Scotia Environment for agricultural land application; **Class B Biosolids:** the policy applied to wood ash with trace metal concentrations that fall above the ‘maximum acceptable metal concentrations for Class A Biosolids but below those for Class B Biosolids is not clear but, at a minimum, a Land Application Plan would be required. Wood ash with trace metal concentrations that exceed the maximum concentrations for Class B biosolids are ‘not acceptable for land application’(Nova Scotia, 2004)
- **CM1:** wood ash with a ‘content of regulated metals’ (CM) that falls below the limits for CM1 non-aqueous non-agricultural source materials (NASM) set by Ontario Regulation 267/03 of the Nutrient Management Act (Government of Ontario 2002); **CM2:** wood ash with a ‘content of regulated metals’ (CM) that falls above the limits for CM1 NASM but below the limits for CM2 NASM. The rules for applying NASM that fall within the limits for CM1 and CM2 NASM are different (e.g., minimum depth to groundwater, proximity to surface water). If the concentrations of one or more trace metals exceed the limits for CM2 materials, then ‘the material cannot be land applied as a NASM’.
- **C1:** wood ash with a ‘chemical contaminant content’ that falls below the limits for category 1 (C1) fertilizing residuals (FR) according to the guidelines described in Hébert (2008) and Hébert (2015); **C2:** wood ash with a ‘chemical contaminant content’ that falls above the limits for C1 FR but below the limits for category 2 (C2) FR. The rules for applying FR that fall within the limits for C1 and C2 materials are different (e.g., maximum application rate). **Note:** If trace metal concentrations do not meet the limits

for C1 or C2 FR, alternative criteria (based on neutralizing value) may be applied to C2 FR used as amendments on forest soils. This option is based on standards set by the Bureau de Normalisation du Québec (BNQ 0419-090). Environment Québec (2004b)

- For agricultural use, the cadmium limit for C2 FR is 10 mg kg-1; for non-agricultural use, the cadmium limit for C2 FR is 15 mg kg

Appendix 4.CFIA fertilizer and supplement metals standards and examples of maximum acceptable metal concentrations based on annual application rates **(Canadian Food Inspection Agency, 2017)**

Metal	Maximum Acceptable Cumulative Metal Additions to Soil over 45 Years (kg /ha)	Maximum Acceptable Product Metal Concentration Based on Annual Application Rates (mg metal/kg product) 4400 kg /ha-yr	Maximum Acceptable Product Metal Concentration Based on Annual Application Rates mg metal/kg product) 500 kg /ha-yr
Arsenic (As)	15	75	666
Cadmium (Cd)	4	20	177
Chromium (Cr)	210	1060	9333
Cobalt (Co)	30	151	1333
Copper (Cu)	150	757	6666
Mercury (Hg)	1	5	44
Molybdenum (Mo)	4	20	177
Nickel (Ni)	36	181	1600
Lead (Pb)	100	505	4444
Selenium (Se)	2.8	14	124
Thallium (Tl)	1	5	44
Vanadium (V)	130	656	5777
Zinc (Zn)	370	1868	16444

Appendix 5. One-sample statistics – Heavy metal concentration in fly-ash

	N	Mean	Std. Deviation	Std. Error Mean
Cr	12	129.823351	13.3539891	3.8549646
Ni	12	109.902072	16.1754382	4.6694468
Co	12	19.220859	1.2559225	.3625536
Cu	12	195.078661	7.5988960	2.1936123
Zn	12	1061.62604	84.2943106	24.3336715
As	12	4.905955	.6869167	.1982958
Cd	12	.934357	.0712627	.0205718
Pb	12	12.668349	.9057111	.2614563
Mo	12	5.600083	1.6743070	.4833308
Hg	12	.210083	.0609492	.0175945

Appendix 6. One-sample test – Heavy metal concentration in fly-ash comparison with CCME compost A

	t	df	Test Value	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Cr	-20.798	11	210	.000	-80.1766493	-88.661369	-71.691929
Ni	10.259	11	62	.000	47.9020720	37.624689	58.179455
Co	-40.764	11	34	.000	-14.7791411	-15.577116	-13.981166
Cu	-93.417	11	400	.000	-204.9213386	-209.749447	-200.093230
Zn	14.861	11	700	.000	361.6260450	308.067995	415.184095
As	-40.818	11	13	.000	-8.0940447	-8.530491	-7.657599
Cd	-100.412	11	3	.000	-2.0656432	-2.110921	-2.020365
Pb	-525.257	11	150	.000	-137.3316507	-137.907112	-136.756189
Mo	1.242	11	5	.240	.6000833	-.463721	1.663887
Hg	-33.528	11	0.8	.000	-.5899167	-.628642	-.551191

Appendix 7. One-sample test – Heavy metal concentration in fly-ash comparison with CCME compost B

	One-Sample Test						
	Test Value = 1060						
	t	df	Test value	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Cr	-241.293	11	1060	.000	-930.1766493	-938.661369	-921.691929
Ni	-15.012	11	180	.000	-70.0979280	-80.375311	-59.820545
Co	-360.717	11	150	.000	-130.7791411	-131.577116	-129.981166
Cu	-913.982	11	2200	.000	-2004.9213386	-2009.749447	-2000.093230
Zn	-31.166	11	1850	.000	-758.3739550	-811.932005	-704.815905
As	-353.482	11	75	.000	-70.0940447	-70.530491	-69.657599
Cd	-926.787	11	20	.000	-19.0656432	-19.110921	-19.020365
Pb	-1863.913	11	500	.000	-487.3316507	-487.907112	-486.756189
Mo	-29.793	11	20	.000	-14.3999167	-15.463721	-13.336113
Hg	-272.239	11	5	.000	-4.7899167	-4.828642	-4.751191

Appendix 8. One-sample test summary– Heavy metal concentration in fly-ash comparison with CCME soil quality guidelines

	t	df	Test value	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Cr	17.075	11	64	.000	65.8233507	57.338631	74.308071
Ni	13.899	11	45	.000	64.9020720	54.624689	75.179455
Co	-57.313	11	40	.000	-20.7791411	-21.577116	-19.981166
Cu	60.211	11	63	.000	132.0786614	127.250553	136.906770
Zn	35.409	11	200	.000	861.6260450	808.067995	915.184095
As	-35.775	11	12	.000	-7.0940447	-7.530491	-6.657599
Cd	-22.635	11	1.4	.000	-.4656432	-.510921	-.420365
Pb	-219.278	11	70	.000	-57.3316507	-57.907112	-56.756189
V	-79.494	11	130	.000	-72.5236667	-74.531668	-70.515665
Hg	-363.176	11	6.60	.000	-6.3899167	-6.428642	-6.351191

Appendix 9. Variation of soil pH along with biochar addition - Descriptive

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.00	3	6.67333	.015275	.008819	6.63539	6.71128	6.660	6.690
2.50	3	6.96333	.011547	.006667	6.93465	6.99202	6.950	6.970
5.00	3	7.05667	.005774	.003333	7.04232	7.07101	7.050	7.060
10.00	3	7.37667	.005774	.003333	7.36232	7.39101	7.370	7.380
Total	12	7.01750	.262267	.075710	6.85086	7.18414	6.660	7.380

Appendix 10. Summary of ANOVA table - Variation of soil pH along with biochar addition

	Sum of Squares	df	Mean Square	F	Sig.
Treatments	.756	3	.252	2325.410	.000
Error	.001	8	.000		
Total	.757	11			

Appendix 11. Heavy metal concentration in *Trifolium pratense* (above ground biomass) under different % biochar treatments - Descriptives

Element	Biochar %	N	Mean (mg kg^{-1})	Std. Deviation	Std. Error	Minimum (mg kg^{-1})	Maximum (mg kg^{-1})
Cr	0	3	2.31	0.34	0.20	2.10	2.72
	2.5	3	1.58	0.03	0.02	1.55	1.62
	5	3	1.08	0.15	0.08	0.95	1.25
	10	3	0.33	0.04	0.02	0.30	0.39
Ni	0	3	1.93	0.03	0.02	1.89	1.96
	2.5	3	1.47	0.17	0.10	1.31	1.66
	5	3	1.14	0.05	0.03	1.08	1.20
	10	3	0.62	0.05	0.03	0.56	0.66
Co	0	3	0.58	0.07	0.04	0.51	0.65
	2.5	3	0.48	0.03	0.01	0.46	0.53
	5	3	0.41	0.02	0.01	0.39	0.44
	10	3	0.00	0.00	0.00	0.00	0.00
Cu	0	3	1.23	0.09	0.05	1.14	1.32
	2.5	3	0.93	0.05	0.02	0.91	1.00
	5	3	0.70	0.05	0.03	0.64	0.76
	10	3	0.31	0.04	0.02	0.28	0.37
Zn	0	3	15.00	1.37	0.79	13.50	16.20
	2.5	3	12.86	1.37	0.79	11.31	13.92
	5	3	7.35	0.41	0.24	6.89	7.69
	10	3	4.17	0.07	0.04	4.11	4.26
Pb	0	3	0.89	0.04	0.02	0.85	0.93
	2.5	3	0.76	0.04	0.02	0.73	0.82
	5	3	0.62	0.05	0.03	0.56	0.67
	10	3	0.16	0.02	0.01	0.14	0.20
V	0	3	2.31	0.20	0.11	2.13	2.54
	2.5	3	2.24	0.13	0.07	2.10	2.36
	5	3	1.67	0.26	0.15	1.37	1.88
	10	3	0.57	0.03	0.02	0.55	0.62
Mo	0	3	2.43	0.02	0.01	2.42	2.45
	2.5	3	2.35	0.01	0.01	2.35	2.37
	5	3	2.33	0.02	0.01	2.36	2.35
	10	3	2.23	0.01	0.005	2.22	2.24

As	0	3	0.185	0.001	0.001	0.184	0.186
	2.5	3	0.179	0.001	0.001	0.179	.0180
	5	3	0.177	0.001	0.001	0.176	0.179
	10	3	0.169	0.00	0.000	0.169	0.17
Fe	0	3	4.46	0.03	0.02	4.44	4.49
	2.5	3	4.34	0.04	0.02	4.32	4.39
	5	3	4.29	0.03	0.02	4.25	4.32
	10	3	4.10	0.02	0.01	4.08	4.11

Appendix 12. Summary of ANOVA table – heavy metal concentration in *Trifolium pratense* (above ground biomass)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cr	Treatments	6.258	3	2.086	55.875	.000
	Error	.299	8	.037		
	Total	6.557	11			
Ni	Treatments	2.748	3	.916	96.626	.000
	Error	.076	8	.009		
	Total	2.824	11			
Cu	Treatments	1.357	3	.452	110.167	.000
	Error	.033	8	.004		
	Total	1.390	11			
Co	Treatments	.592	3	.197	109.939	.000
	Error	.014	8	.002		
	Total	.606	11			
Pb	Treatments	.894	3	.298	147.890	.000
	Error	.016	8	.002		
	Total	.910	11			
Zn	Treatments	221.995	3	73.998	74.556	.000
	Error	7.940	8	.993		
	Total	229.935	11			
V	Treatments	5.790	3	1.930	58.423	.000
	Error	.264	8	.033		
	Total	6.054	11			
Mo	Treatments	.061	3	.020	112.957	.000

	Error	.001	8	.000		
	Total	.062	11			
As	Treatments	.000	3	.000	112.957	.000
	Error	.000	8	.000		
	Total	.000	11			
Fe	Treatments	9.540	3	3.180	9.190	.006
	Error	2.768	8	.346		
	Total	12.308	11			

Appendix 13. Heavy metal concentration in *Phleum pratense* L(above ground biomass) under different % biochar treatments. Mean values are presented with standard errors, minimum and maximum. - Descriptives

Elements	Biochar %	N	Mean (mg kg^{-1})	Std. Deviation	Std. Error	Minimum (mg kg^{-1})	Maximum (mg kg^{-1})
Cr	0	3	1.79	0.17	0.10	1.67	1.99
	2.5	3	1.59	0.03	0.02	1.55	1.62
	5	3	0.87	0.15	0.08	0.74	1.04
	10	3	0.21	0.01	0.00	0.20	0.22
Ni	0	3	1.98	0.23	0.13	1.75	2.21
	2.5	3	1.46	0.10	0.06	1.39	1.58
	5	3	1.15	0.08	0.04	1.08	1.25
	10	3	0.70	0.17	0.09	0.56	0.90
Co	0	3	0.53	0.01	0.00	0.52	0.55
	2.5	3	0.39	0.03	0.02	0.37	0.44
	5	3	0.38	0.00	0.00	0.37	0.39
	10	3	0.00	0.00	0.00	0.00	0.00
Cu	0	3	1.25	0.04	0.02	1.20	1.28
	2.5	3	0.75	0.02	0.01	0.73	0.78
	5	3	0.63	0.01	0.00	0.62	0.65
	10	3	0.27	0.01	0.00	0.27	0.29
Zn	0	3	9.94	1.56	0.90	8.59	11.66
	2.5	3	8.04	0.16	0.09	7.88	8.20
	5	3	5.55	0.41	0.24	5.09	5.89
	10	3	3.60	0.37	0.21	3.20	3.95
Pb	0	3	0.70	0.04	0.02	0.66	0.74
	2.5	3	0.57	0.04	0.02	0.54	0.63
	5	3	0.47	0.00	0.00	0.47	0.48
	10	3	0.11	0.01	00.01	0.10	0.13
V	0	3	1.71	0.20	0.11	1.53	1.94
	2.5	3	1.48	0.19	0.11	1.29	1.68
	5	3	1.27	0.19	0.11	1.07	1.46
	10	3	0.22	0.06	0.035	0.16	0.28
Mo	.00	3	2.398	0.041	0.024	2.351	2.426
	2.50	3	2.357	0.011	0.006	2.351	2.370
	5.00	3	2.333	0.018	0.010	2.316	2.351

	10.00	3	2.231	0.008	0.005	2.223	2.239
As	.00	3	.171	.001	.001	.170	.172
	2.50	3	.166	.001	.000	.166	.167
	5.00	3	.165	.001	.001	.163	.166
	10.00	3	.158	.001	.000	.157	.158
Fe	.00	3	3.942	0.096	0.055	3.832	4.011
	2.50	3	3.890	0.031	0.018	3.872	3.926
	5.00	3	3.845	0.026	0.015	3.819	3.872
	10.00	3	3.693	0.012	0.007	3.681	3.705

Appendix 14. Summary of ANOVA table – Heavy metal concentration in *Phleum pratense* L (above ground biomass)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cr	Treatments	4.654	3	1.551	110.503	.000
	Error	.112	8	.014		
	Total	4.766	11			
Ni	Treatments	2.605	3	.868	34.039	.000
	Error	.204	8	.026		
	Total	2.809	11			
Cu	Treatments	1.475	3	.492	628.627	.000
	Error	.006	8	.001		
	Total	1.481	11			
Co	Treatments	.473	3	.158	405.966	.000
	Error	.003	8	.000		
	Total	.476	11			
Pb	Treatments	.571	3	.190	173.727	.000
	Error	.009	8	.001		
	Total	.579	11			
Zn	Treatments	69.608	3	23.203	33.223	.000
	Error	5.587	8	.698		
	Total	75.195	11			
V	Treatments	3.896	3	1.299	42.309	.000
	Error	.246	8	.031		
	Total	4.141	11			
Mo	Treatments	.046	3	.015	28.225	.000
	Error	.004	8	.001		
	Total	.050	11			
As	Treatments	.000	3	.000	112.957	.000
	Error	.000	8	.000		
	Total	.000	11			
Fe	Treatments	.103	3	.034	12.487	.002
	Error	.022	8	.003		
	Total	.125	11			