Wood Ash and Paper Sludge: Potential Liming and Nutrient Source for crop production in Podzolic Soils

By

Bilal Javed

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

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Abstract

The Corner Brook Pulp and Paper Ltd (CBPPL) produces approximately 10,000 Mg of wood ash (WA) and 47,500 Mg of paper sludge (SL), annually, as waste byproducts. Among these wastes WA is landfilled as part of the disposal management program administered by the company whereas SL is burnt to generate steam for paper mill operations. This practice is not ideal due to high disposal costs and environmental concerns. In addition, WA and SL contains essential nutrients such as Ca, K and Mg that favors plant growth and could increase soil pH due to its calcium carbonate equivalent ability. One sustainable management practice is the land application of WA and SL to agricultural soil, increasing soil pH and enhancing crop growth and yield. Herein, we conducted greenhouse studies to evaluate the effect of WA and SL alone and in combination with biochar (BC) on growth, yield, nitrogen dynamics, and heavy metals mobility in the soil-plant system of annual ryegrass (*Lolium perenn*e L.) and kale (*Brassica oleracea* L.). In study 1 soil with pH 5.7 and 2.7 % organic matter was used and in study 2, soil pH and organic matter were 5.2 and 4.5 % respectively. Results of the study 1 and 2 indicated that WA was effective in achieving the target soil pH of 6.3 and produced 71% and 42% more biomass than control in annual ryegrass, respectively. Similarly, WA application showed 28% and 27% higher yield than control in kale in both studies, respectively. BC addition significantly decreased 15 – 20 % Pb and Cd uptake in annual ryegrass and kale crops in both studies. Study results suggest that WA could be used as a liming and nutrient source and could be a substitute for limestone used in improving soil pH in agriculture production system. Furthermore, BC amendment with WA or SL could be a promising strategy in decreasing heavy metals uptake in agronomic and horticultural crops.

General summary

Highly acidic, poor fertile soils and extreme weather conditions of Newfoundland and Labrador (NL) are major constraints to agricultural production. The Corner Brook Pulp and Paper Ltd. (CBPPL) produces wood ash (WA) and paper sludge (SL) as two waste byproducts, during the paper manufacturing process. These waste byproducts could be potential liming and nutrient sources due to high pH and availability of essential minerals for plant growth. Greenhouse experiments showed that application WA alone and in combination with biochar (BC) enhanced soil pH, growth and yield of annual ryegrass and kale. Whereas heavy metals concentration in WA and SL were below the allowable limits set by Canadian Council of Ministers of the Environment (CCME). However, application of WA and SL along with BC decreased uptake of heavy metals by annual ryegrass and kale and increased their concentration in soil. WA and SL has liming and agronomic benefits and could be used as a liming and nutrient source in agriculture production system.

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Co-authorship statement

Manuscripts based on the chapter 2, entitled "*Evaluating the effects of wood ash, paper sludge and biochar on growth, yield, soil nitrogen dynamics and heavy metals uptake in annual ryegrass and kale*" and Chapter 3, "*Evaluating paper mill biosolids and biochar on growth, yield, and heavy metals mobility in a low pH soil-plant system*" will be submitted to Journal of Agronomy (Javed, B., Katanda, Y., Galagedara, L., Thomas, R., Guo, X., Farhain, M.M., Wickremasinghe, T., Nadeem, M., Cheema, M.) and Science of the Total Environment (Javed, B., Katanda, Y., Galagedara, L., Thomas, R., Guo, X., Farhain, M.M., Wickremasinghe, T., Nadeem, M., Cheema, M.), respectively. Bilal Javed, the thesis author will be the primary author and Dr. Mumtaz Cheema (supervisor), will be the corresponding and last author. Dr. Lakshman Galagedara (co-supervisor), Dr. Raymond Thomas and Dr. Xiaobin Gou (committee member) will be third, fourth and fifth authors, respectively.

For work in Chapter 2 and Chapter 3, Mr. Bilal (designed and laid out experiments, collected data and statically analyzed and drafted the manuscript). Dr. Mumtaz Cheema (supervisor), got research funds, designed experiment, helped in data analysis, results interpretation, and edited the manuscript. Dr. Yeukai Katanda assisted in experimental set up, data collection, analyses, review, editing and interpretation of this thesis. Dr. Lakshman Galagedara helped in results explanation and reviewed and edited the thesis. Dr. Raymond Thomas and Dr. Xiaobin Guo (committee members) helped to finalize the methodology, data analysis/interpretation, edited and reviewed the manuscript. Dr. Muhammad Nadeem helped in statistical analyses, results interpretation and edited the manuscript, whereas Muhammad Mashallah Farhain and Thilini helped in data collection.

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List of abbreviations

WA = Wood ash

SL = Paper Sludge

BC = Biochar

CCE = Calcium carbonate equivalent

CBPPL = Corner Brook Pulp and Paper Ltd.

CEC = Cation Exchange Capacity

EC = Electrical Conductivity

ICP-MS = Inductively Coupled Plasma Mass Spectrometry

RDA = Redundancy analysis

Chapter 1

General introduction and review of literature

1.1 Introduction

Newfoundland and Labrador (NL) is the 16th largest island in the world with an area of 405,212 square kilometers. This province is located in the most easterly part of Canada and at the north-eastern corner of North America. NL is uniquely situated in a geographical area with extreme weather conditions characterized by short growing season, and low fertile soils (podzolic) which restricts crop growth, development, and yield. As such, only 10% food consumed in the province is locally produced, with the remainder imported from mainland Canada and other countries e.g., United States (Department of Fisheries, Forestry and Agriculture 2017). There is a dire need to improve soil fertility, pH, and health through efficient utilization low cost inputs to achieve food self-sufficiency without impacting environment. Though NL is self-sufficient in poultry and milk production, the industry heavily depends on imports of forages/silage from other provinces to support the animal feed needs of the livestock sector in the province (Reza, 2019; Statistics Canada, 2017).

Newfoundland soils are mostly podzolic in nature and cover more than 60% of the province's total area. Podzols are darker alluvial soils having high levels of aluminum (Al^{3+}), iron (Fe^{2+}) ions and organic matter in the B horizon. (Sanborn et al., 2011). Dissolved Al^{3+} in these soils could be toxic and may inhibit root growth by damaging the root apical cells affecting the plant's uptake of nutrients and water, resulting poor yield (Kopittke et al., 2015; Yamamoto, 2019). In addition, podzolic soils have high percentage of clay minerals containing kaolinite and iron oxides which can fix phosphorous (P) and make it unavailable for plant uptake. Thus low pH, excessive Al^{3+}, and unavailability of P are the main causes of low agriculture production in podzols (Truskavets'kyj et al., 2018; Zheng, 2010). Raising soil pH, fertility and health are key factors to enhance crop productivity in podzolic soils. Limestone and inorganic fertilizer applications can raise soil pH and improve soil fertility. However, this can result in a substantial increase in cost of production. There is a need to find low cost inputs such as waste byproducts that can be used as a liming agent and nutrient source to enhance crop production.

Paper mills burn a tremendous amount of wood sludge as energy sources during operation and subsequently produce a higher quantity of wood ash (WA) as a waste bi-product. Corner Brook Pulp and Paper Ltd. (CBPPL) produce approximately 10,000 Mg of WA and 47,500 Mg of paper sludge (SL) annually as waste by-products. WA is disposed at landfill site causing negative environmental impacts and financial implications for the paper industry whereas SL is burnt to generate steam for paper mill operations. This situation could be worst in the future due to increased disposal costs at landfill sites and possibly new environmental legislations governing greenhouse gas emissions and nutrient leaching (Canada, 2017; Lou & Nair, 2009; Ryan, 2009). Sustainable management of WA and SL represents a suitable strategy to protect the environment as well as to minimize the financial burden on the paper industry. Different studies suggest that WA and SL contain macro and micronutrients required for plant growth and hence could be used as soil amendments (Manirakiza et al., 2020; Symanowicz et al., 2018). For instance, WA application increased soil pH, Ca and Mg concentrations and growth of sugar maple trees (Arseneau et al., 2021; da Costa et al., 2020; Pitman, 2006). Bonfim-Silva et al. (2017) reported that WA application enhanced biomass of *Brachiaria brizantha* by 94% compared to control.

Similarly, SL is a rich source of organic matter, and plant essential nutrients (N, P and Ca) (Fahim et al., 2019). Ríos et al. (2012) observed that SL amendment increased organic matter and P content in the soil, consequently increasing biomass production of *Lolium perenne* L. Therefore, WA and SL amendment in acidic soils could be a promising strategy to raise soil pH and soil fertility to enhance plant growth and yield in podzolic soils.

In addition, WA may contains heavy metals due to utilization of used oil in the combustion process (Lucchini et al., 2014). Heavy metals including chromium (Cr), nickel (Ni), arsenic (As), copper (Cu), lead (Pb) and cadmium (Cd) are non-degradable elements, that can persist in soil for a longer time and can negatively affect plant growth and human health (Nicholson et al., 2006; Ozolinčius & Varnagirytė, 2015). These heavy metals could have high impact on the plant internal mechanism as some of the heavy metals are an integral part of the many important processes such as disturbance in the cell wall structure, depletion of cell structure and inhibit the functions of various enzymes which play significant role in the protein synthesis in the plants. Essential metals such as copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), and cobalt (Cb) are required in different plant functions in minute quantities (Arif et al., 2016). When these metals are bioavailable in excessive quantities, they cause toxicity to the plants. For example, Plants growing on metal-contaminated soils have altered their mechanism due to cell toxicity (Polle & Schützendübel, 2003).

There is need to reduce the heavy metal uptake in growing plants as well as the heavy metal contamination risks in the food chain. One of the available techniques is the use of organic soil amendments which can reduce the mobility of heavy metals in the soil-plant system (Komárek et al., 2013). Biochar (BC) is a black carbon (C) or solid material obtained from the thermochemical conversion of biomass (pyrolysis of the waste residues from forestry, crops and manure) in an oxygen-limited environment (Chen et al., 2018; Xu et al., 2013). BC is a stable solid, C rich soil amendment that can be used for C sequestration and soil health benefits and can endure in soil for thousands of years (Lu et al., 2012). Additionally, BC types have various capacities in the adsorption and sorption of heavy metals. BC features are dependent on the pyrolysis conditions such as temperature, residence time, moisture content of the feedstock, and the type of feedstock used in pyrolysis. BC has high pH and cation exchange capacity (CEC) and therefore can increase soil productivity (Beesley et al., 2011). We hypothesized that WA, SL and BC application would raise soil pH, improve crop growth, yield, N uptake and reduce heavy metals uptake in annual ryegrass and kale. To test the hypothesis, we conducted a greenhouse experiment with the following specific objectives:

i. To investigate the effects of WA, SL, WA + SL alone and in combination with BC on seedling emergence, chlorophyll, photosynthesis, and yield of annual ryegrass and kale.

ii. To examine the effects of WA, SL, WA + SL, alone and in combination with BC on soil pH, N uptake and heavy metals uptake in annual ryegrass and kale.

1.2 Review of literature

1.2.1 Podzolic soils

The name podzol was derived from a Russian term “pod” means under and “zol” means ash. This term was first used by Russian workers for ash grey horizon located near the surface of many soils (Mokma & Buurman, 1987).These are forested soils formed of sandy parent material, underlying by igneous rocks. Podzolic soils covers 14.3 % of the overall Canadian land which can be found in two different areas, British Columbia and eastern Canada (northern Ontario, Quebec, Maritimes) mainly in the boreal forest regions (Sanborn et al., 2011). In the Atlantic Provinces, podzolic soils have fine particles (sandy soils) occupies about 55 to 80 % of the Appalachian region (Browne & Davis, 2009). In NL, 55.2 % (209,377 km^2) of the landmass is covered by podzolic soils of which 44.2 % (92,533 km^2) is Fero-humic and 55.8 % (116,843 km^2) is of the Humo-Feric Podzolic group (Ecological Stratification Working Group, 1995). Podzols are formed by a series of processes called podzolization.

Podzols evolved based on soil-forming properties such as climate, vegetation, topography, parent material and age of the soil. The sandy sediment acquired from igneous rocks parent material naturally has an acidic pH because of the mineralogical properties. Coniferous plants are the major type of vegetation found on the podzolic soils (Burton et al., 2003). Due to the presence of the vegetation, plants shed their leaves on the soil surface causing increased acidity in the upper layer of the soil, which is further increased by organic decomposition of leaf litter (Lopes-Mazzetto et al., 2018). This process creates a robust chemical weathering zone in the upper layer of the soil where minerals such as aluminum, iron and other metal ions become bioavailable and released into the soil solution. In the soil solution these metallic ions form complex compounds with the organic decomposition products called chelates. These compounds move vertically with draining water into the B horizon and get deposited. This overall process is known podzolization (Lundström et al., 2000).

Podzols have low pH as compared to the other soil orders due to its parent material, climate and vegetation effect (Engelen et al., 2008). Podzols tend to have low pH buffering capacity due to the vegetation that produces large numbers of organic acids and climates where annual rainfall is more than its evapotranspiration. Natural weathering causes significant leaching of organic products down into the soil profile (Sauer et al., 2007). Higher amount of dissolved organic acids and low pH of the soil solution provides an environmental condition that promotes the weathering of the soil minerals (Jersak et al., 1995). Low pH of acidic soils is due to hydrogen ions (H^+) in the soil, deposited by the root exudates, atmospheric deposition, and weathering of the minerals. In Podzols, several processes transfer the acidity down into the soil profile. The protonated organic acids move from the upper layer of the soil to the lower layers by leaching and may release hydrogen ions into the soil solution. Al^{3+} is released due to weathering in the uppermost layer of the soil and leached down the soil profiles as hydrated Al^{3+} and act as the primary cause of acidity and root toxicity (Hendershot et al., 1991; Kopittke et al., 2015; Yamamoto, 2019). Due to the acidic nature or low pH of podzolic soils, liming is done to raise the soil pH to levels that favors plant nutrients availability and enhance crop growth, development, and yield.

1.2.2 Liming

Newfoundland soils are acidic thus require liming to neutralize soil acidity. Liming increases the soil pH, improves the nutrients availability, plant uptake, and increases the bacterial to the fungal ratio of the soil (Bothe, 2015; Holland et al., 2018). The practice of lime applications changes the chemical balance in the soil. Most of the liming material has Ca, Mg or both cations and they neutralize the acidic effect by replacing the hydrogen ions (H^+) in the soil solution (Kowalenko & Ihnat, 2010). In case of limestone, the reaction is described as:

$$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + CO_2 + H_2O \tag{1}$$

The above reaction explains the neutralization processes that describes the effect of liming on soil chemical properties. Limestone ($CaCO_3$) reacts with hydrogen ions, forms a calcium cation (Ca^{2+}), CO_2, H_2O and this chemical reaction continues until all the lime has reacted (Anderson et al., 2013). There are 2 moles of acids that are consumed for each mole of liming material. In the above reactions, CO_2 evolved which shows the liming impact on the carbon cycle. The practice of liming for neutralizing the soil acidity has significant impact on the biogeochemical cycles of C, N, and sulfur (S) (Fornara et al., 2011). Soil amendments such as WA and SL could be used as liming material instead of limestone on the basis of calcium carbonate equivalent (Griffin, 2004). Calcium carbonate ($CaCO_3$) equivalent (CCE) is the measure of the amount of acid that can be neutralized with a liming material compared to pure $CaCO_3$ (Mullins et al., 2019). Furthermore, calcium carbonate equivalent can be measured following the dissolution of calcium carbonate with acid and either the CO_2 evolved or acid consumed is estimated (Moore et al., 1987).

1.2.3 Wood ash and paper mill sludge as growing media amendments

Growing media amendments are of either plant or animal origin and can be utilized in the soil to improve the physical properties such as water-holding capacity, porosity, bulk density, water infiltration, and structure. Furthermore, organic amendments can add soil organic carbon as well as can improve soil chemical properties including cation exchange capacity (CEC), EC and pH respectively (Sutton-grier et al., 2009; Wang et al., 2014). Besides that, organic amendments also add nutrients to the soil through mineralization process depending on the C/N ratio. WA and SL are the most abundant wastes produced by paper manufacturing industries. Previous studies have revealed potential of WA and SL amendment in agriculture and forestry as liming and nutrient sources. WA could be used as a liming material, and it is a convenient way to recycle the nutrients back into the soil (Risse & Gaskin, 2010). WA and SL increase the organic matter content in the soil, which improves soil quality, e.g., provides better aeration, increases water holding capacity, prevents leaching of the essential nutrients, and promotes microbial growth that improves the overall health of the soil (Cooperband, 2002). The addition of WA and SL may help improve soil quality and health to boost agriculture production.

1.2.4 Wood ash and paper mill sludge as liming and nutrient sources

1.2.4.1 Wood ash

Wood ash (fly ash + bottom ash) is the residue that settles at the bottom of the furnace during high-temperature combustion of wood, bark, and other organic residues. Different types of wood ash (bottom ash and fly ash) are composed of various amounts of calcium, magnesium, sodium, silicon, iron, and manganese with heavy metals as contaminants (Österås et al., 2005). Ash produced as a result of the combustion process is collected together and exported to landfill sites as waste products (Hawrot et al., 2017). However, this waste by product can raise soil pH as a liming source and provide essential nutrients to the soil. The liming and nutrient properties of WA depends upon many factors. These include plant species and part of the plant which is used in the combustion process (leaves, bark wood), fuel source used in the combustion process and storage conditions of the ash (Someshwar, 1996). The application of WA back to the soil is the most sustainable way for its utilization. This approach can ensure the macro and micronutrients taken by the plants return to the soil, completing the mineral cycle (Kajda-Szcześniak, 2014). Several previous studies suggested that WA could be used as a liming and nutrient source and increased crop yield. WA could act as a liming source due to the presence of $CaCO_3$ that neutralizes (H^+) which present in low pH soil with high concentration (Scheepers & Toit, 2016). Sharifi et al., (2013) reported, WA application increased soil pH in two different types of acidic soils with pH (4.3 and 5.4) and stabilized soil pH for eight months. The neutralizing potentials for WA can range between 8-90 % (Risse & Gaskin, 2002). Yang et al., (2018) reported pH increases in loamy sand soil that had received WA under incubation conditions. Vance., (1996) reported a range based on calcium carbonate equivalent between 13.2 % and 92.4 %, with average of 48.1 % of WA samples from 18 different wood ash boiler samples. The variation observed was due to the combustion temperature and storage period as the alkalinity of the WA decreases with increasing temperature during the combustion and storage time.

WA is known to be a good source of macronutrients such as potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), and micronutrients including iron (Fe), copper (Cu), zinc (Zn) and molybdenum (Mo) (Adekayode & Olojugba, 2010; Demeyer et al., 2001; Kukier & Sumner, 1996; Naylor & Schmidt, 1986; Saarsalmi et al., 2001; Sharifi et al., 2013). WA has the potential to enhance the bioavailability of plant nutrients by raising soil pH (Hakkila, 1989; Mbah et al., 2010; Naylor & Schmidt, 1986; Ohno & Erich, 1990; Sharifi et al., 2013a).

Maschowski et al., (2016) reported WA contains 50 % K by weight and can be used as K fertilizer source. Soils treated with WA have shown an increase in pH and nutrients and a decrease in the movement of the toxic elements (heavy metals) (Ochecova et al., 2014). Several studies reported that WA application increased crop growth and yield. For example, Kikamägi et al., (2013) reported, WA application increased *betula pendula* height compared to limestone. Similarly, WA application at the rate of 2.5 t ha^{-1} increased growth and produced 46% and 28 % more soybean yield at two different experimental sites (Soretire and Olayinka, 2012). In addition, WA may contain heavy metals due to utilization of used oil in the combustion process. Used oil contains a different type of heavy metals that may be volatile and condensed simultaneously as part of the WA (Obernberger et al., 2006). These heavy metals can be adsorbed into the biomass at minute concentrations during normal growth of the plants but may become toxic when present in higher quantities (Nzihou & Stanmore, 2013).

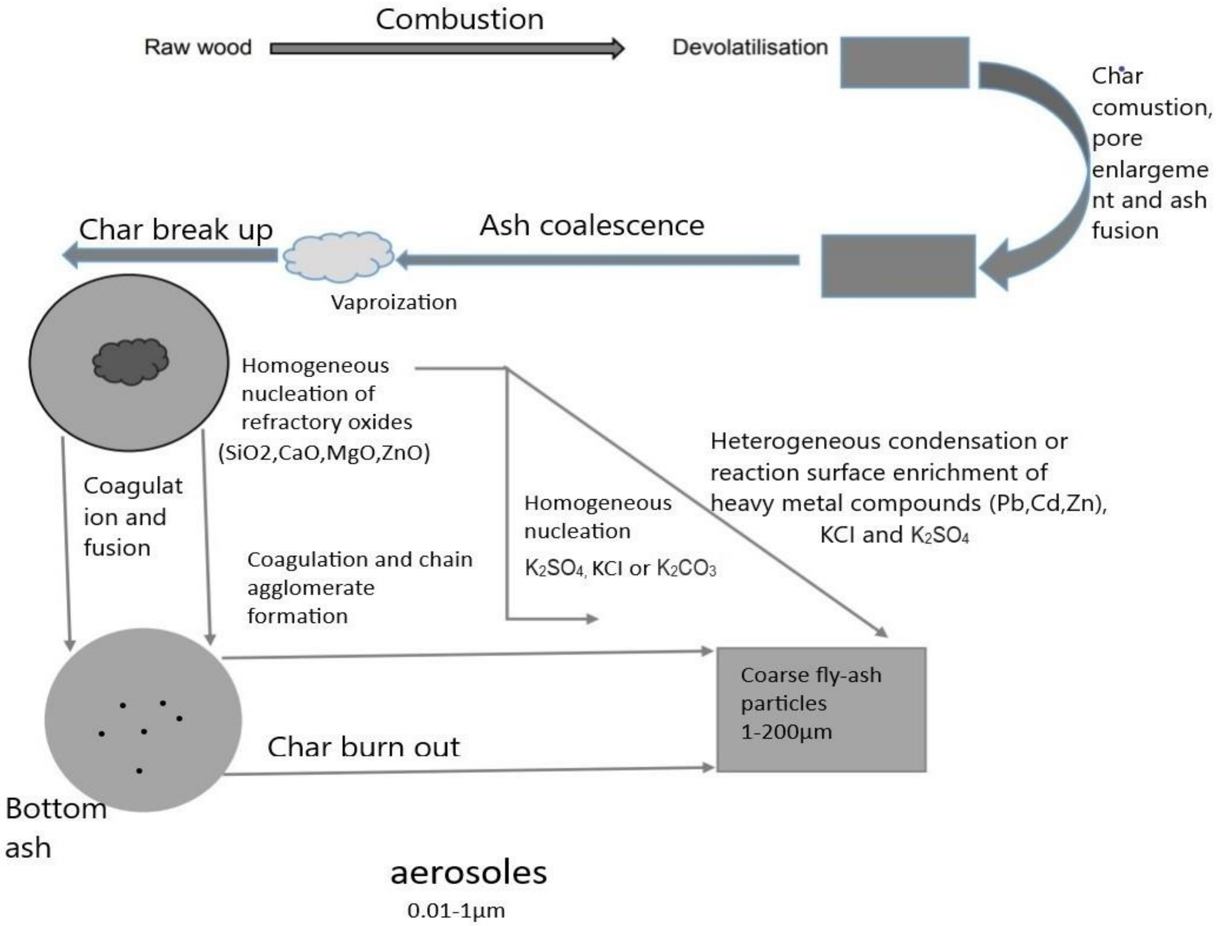

Figure 1.1: Schematic diagram of ash formation from biomass by combustion process (Obernberger et al., 2006).

During the combustion process at the first step is the devolatilization of the woody particles take place and biomass is converted into the char, which combines the oxygen, nitrogen, and hydrogen. In the next step, coalescence of ash takes place. At this point, vaporization of heavy metals with lower molecular weight, such as Pb, Cd, and Zn, tends to vaporize and re-condense into fine particles (1-200 µm), called coarse fly ash. On the other side, oxides such as SiO_2, CaO, MgO, ZnO) vaporized and becomes the part to form char. After ash coalescence, char is further breakdown and at this stage, coagulation and fusion of the char particles take place to form bottom ash. Those heavy metals which do not vaporize during ash coalescence follow the char path and then turn into the bottom ash (Figure 1.1). An enrichment factor determines the portioning of the heavy metals in the wood ash.

The enrichment factor is the ratio of the quantity of elements in the ash from the boiler to its effective concentration in the original fuel. These heavy metals are classified into three different classes. Class-I is with the least volatile elements, and Class III has the most volatile heavy metals. Class-II is the intermediate class that is further divided into three sub classes: a, b and c. Class-I include Ca, Cu, Cr, Fe and Ni and are the least volatile becoming part of bottom ash. Class-III metals include selenium, mercury and boron that is typically vaporizing resulting in small amounts in the ashes. The enrichment factor in the ashes increases as the particle size of the ash decreases, because of the higher area per unit mass. Only selenium and mercury leave the combustion system in the vapor form as HgO and oxidized form as $HgCl_2$ (Nzihou & Stanmore, 2013; Obernberger et al., 2006).

1.2.4.2 Paper sludge

Paper sludge (SL) is a term used for the effluent wastes generated from pulp and paper production. Sludge is produced at two different stages in the process of treating effluent. In first step effluent is passed through the primary clarifier. Primary sludge is recovered after processing at the clarifier. The primary clarification is carried out by sedimentation processes and can also be done via dissolved air floatation. In the sedimentation process, wastewater is pumped into the large tanks, where the solids become settled and then removed from the bottom of the settling tanks. Based on the material characteristics, these residues can vary from 1.5% to 6.5%. Top clear water from the tank is then passed to a secondary clarifier for secondary treatment. Secondary treatment mainly comprises biological processes in which microorganisms convert the waste into water and carbon dioxide by consuming oxygen. The residues generated in this process are removed through clarification similar to the primary clarification process. The process of sludge formation can be seen in Figure 1.2.

Landfilling and incineration of the SL are very unfavorable means of disposal. On the other hand, SL could improve soil physio-chemical properties such as water-holding capacity, organic matter percentage, soil pH, aggregate stability and cation exchange capacity (Camberato et al., 2006). It is an excellent source of C thus the addition of SL could increase soil organic C (SOC) that could persist for a long period of time (Mabee & Roy, 2003). SL is composed of high organic matter and minerals such as nitrogen, carbon, phosphorous, calcium carbonates and could be used as liming material for low pH soils. The neutralizing potentials for SL varied between 12 % - 60 % (Kar et al., 2014; Torkashvand, 2010). SL as liming material increased soil pH, calcium, magnesium, neutralization of toxic Al^{3+}, enhanced phosphorous availability and other minerals nutrient uptake and increased crop biomass. Torkashvand (2010) reported SL application increased soil pH from 4.9 - 6.2 and that might be due to higher $CaCO_3$ (32.5%) concentration of the SL. Similarly, increases in soil pH at two different sites was observed with increasing SL application rate (Aitken et al., 1998). Ríos et al. (2012) reported that SL as soil amendment increased organic matter and P content in the soil, consequently increased the biomass of *Lolium perenne* L.

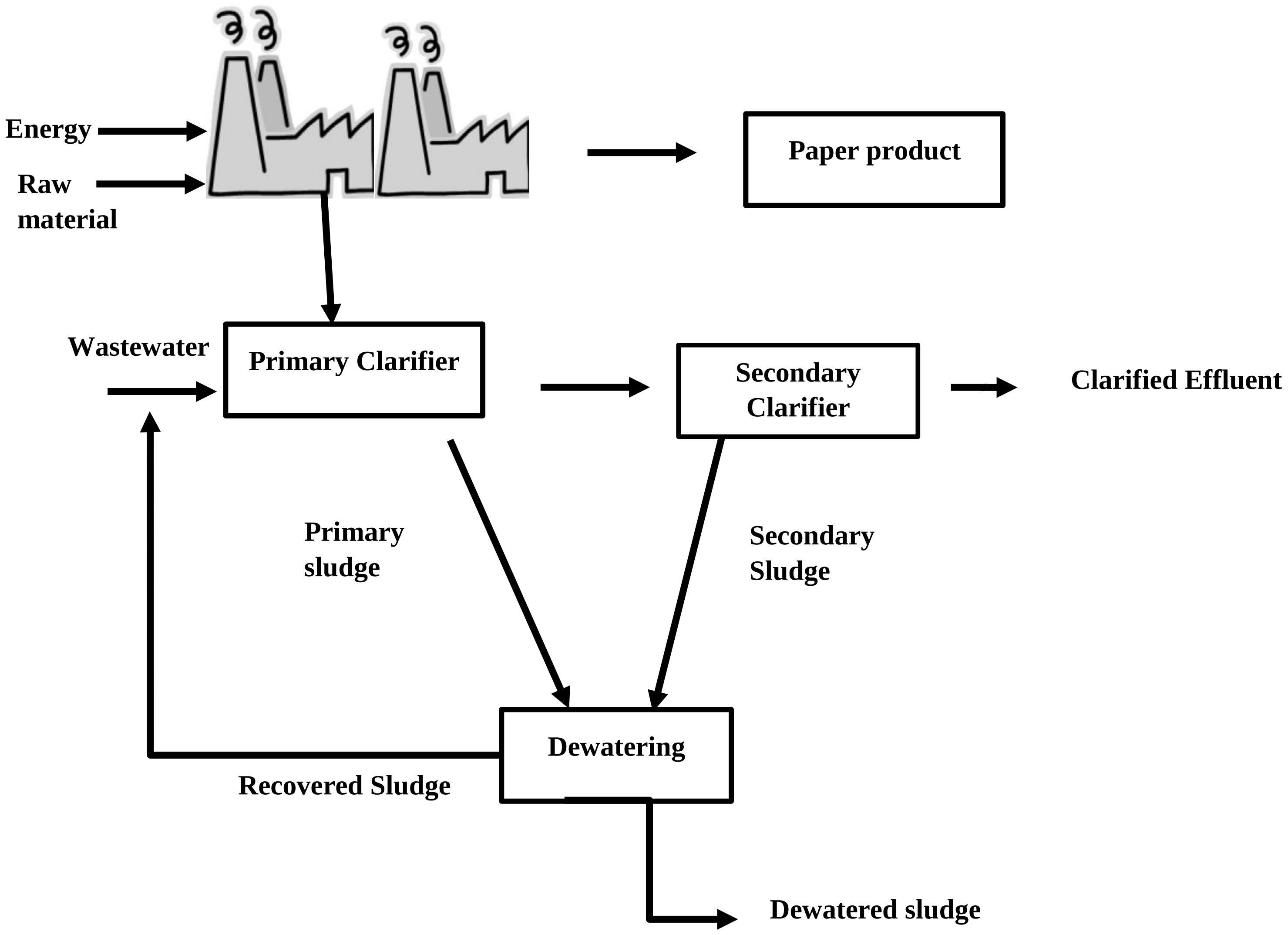

Figure 1.2: Steps involved in the production of paper mill sludge.

Soil amended with SL exhibited increased root length, stem and yield of *Phaseolus vulgaris* L (Kumar & Chopra, 2014). Ziadi et al. (2013), reported paper mill biosolids application in a low pH soil increased N, P, Ca concentration in soil and enhanced crop biomass of *Hordeum vulgare* L. and *Phaseolus vulgaris* L. WA and SL as industrial byproducts may contain heavy metals which could limit their use in crop production, as heavy metals have determinantal effects on plant growth and development (Obernberger et al., 2006).

1.2.4.3 Heavy metals risk and paper mill wastes

Heavy metals are non-degradable elements that are characterized by high density and high atomic weight. Heavy metals such as Co, Cu, Fe, Zn, Ni, and Mo are required in minute quantities (0.03 – 0.05, 6.68 – 24.46, 61.87 - 67.0, 16.11 – 113.8, 0.738 – 6.03, 0.576 – 4.26 mg/kg) respectively, safe for human consumption (Mihaljev et al., 2014); however excess amount of these metals can be harmful for plants and animals (Chibuike & Obiora, 2014). Each heavy metal has the ability to cause oxidative stress in the plants. The mechanism behind heavy metal toxicity in plants is that free oxygen radicals are produced when molecular oxygen accepts electrons from other molecules and is reduced to the reactive oxygen species (ROS) e.g., superoxide (O_2^-) and hydrogen peroxide (H_2O_2). These ROS are responsible for oxidative damage in the plant's internal biological system such as by damaging cell membranes, DNA and other cell structures (Rai et al., 2019). Most of the heavy metals (M) have unpaired electrons, which decreased oxygen availability.

$$M_n + O_2 \rightarrow Mn^{n+1} + O_2^- \tag{2}$$

This oxygen radical at neutral pH in an aqueous solution can produce hydrogen peroxide (H_2O_2), which can further convert into OH^- via Habber-Weiss reaction (Rao et al., 2016).

$$Mn^{n+1} + O_2^- \rightarrow M_n + O_2 \tag{3}$$

$$M_n + H_2O_2 \rightarrow Mn^{n+1} + OH^- + OH \tag{4}$$

These reactions are summarized as

$$O_2 + H_2O_2 \rightarrow O_2 + OH^- + OH \tag{5}$$

These ROS produced in this process cause oxidative stress in plants e.g., hydroxyl ion produced in the proximity of the DNA cause retardation in the cell's normal functions, react with free amino acids and protein and cause damage to their structures (Rao et al., 2016). Heavy metals and their morphological stress on plant growth can be seen in Table 1.1.

Table 1.1: Detrimental effects of heavy metals on plant growth

Heavy metal	Function in plants	Morphological stress	Reference
Essential heavy metals			
Copper	• Promote photosynthesis • Cell wall metabolism • Biogenesis of molybdenum co factor	• Alter root structure by reducing root hair proliferation and root cuticle damage • Reduced crop yield • Chlorosis in leaves	(Kumar et al., 2021; Yruela, 2009)
Iron	• DNA synthesis • Formation of chlorophyll • Maintenance of chloroplast structure	• Leaf discoloration • Stunted root growth • Can damage lipids and proteins structures	(Li et al., 2016; Rout & Sahoo, 2015)
Molybdenum	• Facilitate nitrogen assimilation • Potassium absorption and improves plant growth • Improves plant growth	• Toxicity is very rare • Yellowing of leaves in some cases	(Manuel et al., 2018)
Zinc	• Activate enzymes for protein synthesis • Help plants to withstand cold temperatures • Facilitate formation of chlorophyll and carbohydrates	• Inhabitation in the absorption of essential elements • Inhibits root growth • Inhibits chlorophyll synthesis • Reduce photosynthetic activity in plants	(Broadley et al., 2007; H. Zhang et al., 2020)
Non-essential heavy metals			

Cadmium	• ------	• Root inhibition • Reduction in plant growth and biomass • Decrease photosynthesis activity • Compete with other minerals e.g., Zn, Fe and Ca.	Ismael et al., 2019
Chromium	• ------	• Cause toxicity in seed germination • Reduce stem and root growth • Leaf necrosis and chlorosis • Cause lipid peroxidation	Shahid et al., 2017
Lead	• ------	• Decrease chlorophyll content • Reduce the absorption of essential nutrients by the plants • Changes the structure and permeability of the cell membrane	Venkatachalam et al., 2017
Nickle	• Component of various plant enzymes • Act as catalyst in the enzymes	• Cause leaf chlorosis and production of abnormal shape flowers • Retards seed germination ability • Cause iron deficiency in plants	Rao et al., 2016

1.2.4.4 Role of biochar in remediation of heavy metals in contaminated soils

BC is a C rich product produced from slow thermo chemical pyrolysis of organic residues from agriculture and forestry (Jien & Wang, 2013). It can improve soil physical, chemical and biological properties and facilitates crop growth, carbon sequestration into the soil, and reduce greenhouse gas emissions (Blanco-Canqui, 2017; Brassard et al., 2016; Guo et al., 2015). Many studies have revealed that BC could improve soil physiochemical properties such aggregate stability, maintain soil organic matter and reduce soil erosion (Kimetu & Lehmann, 2010; Tejada & Gonzalez, 2007). There are studies which showed that BC application significantly affected soil chemical properties including soil pH, EC and CEC (Mosharrof et al., 2021; Unger & Killorn, 2011). Similarly, soil amended with BC affects soil biologically stimulating soil microbial biomass and their activity (Gwenzi et al., 2015; Jaiswal et al., 2017; Lehmann & Joseph, 2015). Chen et al. (2020) reported, BC application promotes the growth of beneficial bacteria e.g., *Aeromicrobium*, *Bacillus*, *Bradyrhizobium*.

Heavy metals are non-degradable elements that can persist in soil for longer time than macro-elements, including calcium and magnesium. The practice of removing heavy metals from the soil is expensive and time-consuming. Fixation of heavy metals in the soil by adding amendments such as compost and biochar is an economical way to stabilize heavy metals in the soil than conventional chemical methods (Komárek et al., 2013). BC could be used as a soil amendment to stabilize heavy metals in the soil and hinders the accumulation of heavy metals in the plants (Khorram et al., 2016); Wang et al., 2020). The characteristic of the BC depends on the temperature of the pyrolysis process, type of feedstock used in the BC production, residence time, and moisture content of the feedstock (Zhang et al., 2013).

Lu et al. (2012) proposed three-way mechanisms by which BC stabilize heavy metal in the soil as shown in Figure 1. (heavy metals are indicated as M^{2+}).

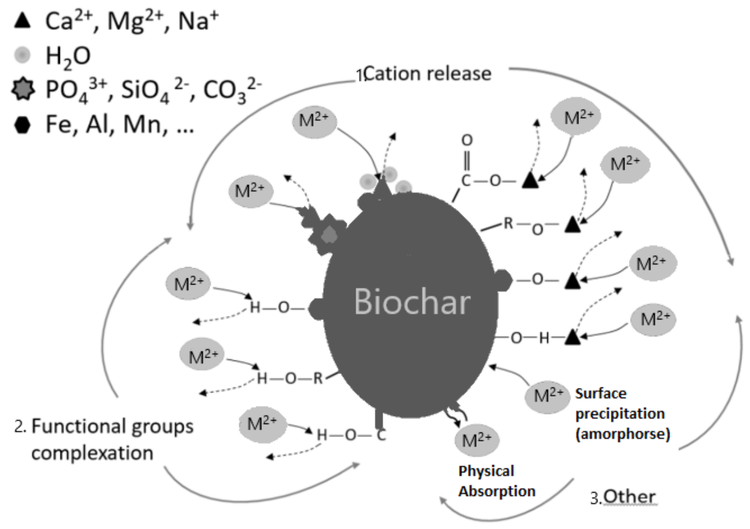

Figure 1.3: Mechanisms of biochar for heavy metal stabilization (Lu et al., 2012).

These three way mechanisms include; (1) heavy metal exchange with cations, e.g., Ca^{2+} via electrostatic cation exchange or metal exchange reactions forming inner sphere complexes with humic matter and mineral oxides present in BC, (2) formation of surface complexes with carboxyl and hydroxyl functional groups functional groups and inner sphere complexation with the free hydroxyl of mineral oxides, for example free carboxylic and hydroxyl functional groups present in humic substances form complexes with metal ions such as ($-COOH + Me^{2+} \rightarrow -COOMe$) whereas Me represent the central metal atom), and (3) surface precipitation and physical absorption of heavy metals (Lu et al., 2012). In addition, BC have highly porous structure containing larger groves that allows it to park heavy metals within these large groves result in sorption of heavy metals (Zhang et al., 2013). Similarly BC contains several functional groups including carboxylic that facilitates the adsorption of heavy metals (Essandoh et al., 2015). Yang et al. (2019) reported, BC has ability to adsorb heavy metals via ion exchange between heavy metals and protons present oxygen containing functional group including carboxylic and hydroxyl groups, however, the efficiency of ion exchange method via functional groups depends upon the size of the ions present on the heavy metal and surface chemistry of the particular functional groups.

In addition, BC derived from sugarcane straw decreased the available Pb, Cd and Zn by 50, 56.5 and 54 %, respectively (Puga et al., 2015). Carbonates and phosphates present in biochar play a significant role in stabilizing the heavy metals in soil because of the precipitation property of salts, which can precipitate with heavy metals and reduce their bioavailability (Cao et al., 2009). Yu et al. (2015) reported improved biochar by manganese oxide and stabilizing arsenic (As) in the soil. The arsenic amount in rice plant was reduced with manganese oxide amended biochar by oxidation of the arsenite to arsenate.

1.3 Thesis organization

The thesis is organized in a manuscript style and divided into four chapters. The thesis has a general introduction chapter, two stand-alone chapters (manuscript format) and the last chapter (chapter four) as general discussion and conclusion.

Chapter one: This is the general introduction chapter of the thesis. It provides the overview with background information, rationale, relevant literature, and objective of the thesis.

Chapter two (study one): The title for this chapter is “Evaluating the effects of wood ash, paper sludge and biochar on growth, yield, soil nitrogen dynamics and heavy metals uptake in annual ryegrass and kale”. Soil used in this study consists of 5.7 pH and 2.7 % organic matter.

Chapter three (study two): The title of the chapter is “Evaluating paper mill biosolids and biochar on growth, yield, and heavy metals mobility in a low pH soil-plant system”. In this study different soil with low pH 5.2 and higher percentage of organic matter 4.7 was used. All the parameters measured in this chapter were similar to chapter 2. However, this study further confirms the efficiency of the WA, SL and WASL alone and in combination with BC in a different soil with distinguished chemical features.

Chapter four: This chapter presents an overall discussion about study findings and conclusion. Also, this chapter provides the recommendations for further studies.

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Chapter 2

Evaluating the effects of wood ash, paper sludge and biochar on growth, yield, soil nitrogen dynamics and heavy metals uptake in annual ryegrass and kale

Abstract

Canadian paper industry produces a significant amount of WA and SL as by-products each year. These waste products are typically disposed of at landfill sites at high transportation cost and poses serious environmental risk to water resources and aquatic ecosystems. Sustainable management of these wastes in an economically viable, socially acceptable, and environmentally sound manner are the most critical challenges of the paper industry. One sustainable management option is utilizing these wastes as soil amendments to increase soil pH and nutrients to enhance crop growth and yield. However, WA and SL may contain heavy metals due to utilization of used oil in the combustion process. Heavy metals are non-degradable elements that may cause toxicity, reduces the crop quality and could lead to food insecurity. Organic amendments such as biochar (BC) can be used to adsorb heavy metals to prevent their uptake by plants. The objective of this study was to evaluate WA, SL alone and in combination with BC as a liming and nutrient source to increase soil pH and crop yield as well as assess heavy metals dynamics in podzolic soil-plant production systems. A greenhouse experiment was conducted in a randomized complete block design with three replications. Experimental treatments were Limestone (Control), WA, SL, WASL without BC addition (0 Mg ha^{-1}) and Control, WA, SL, WASL with addition of (20 Mg ha^{-1}) BC. Annual ryegrass (*Lolium perenne* L.) and kale (*Brassica oleracea*) were used as test crops. Results indicated that application of WA at the rate of 17.25 t ha^{-1} efficiently increased soil pH from 5.7

to the target pH of 6.3 in both crops. In comparison to control, WA treatment produced by 41% and 28% higher biomass/yield of annual ryegrass and kale respectively. Paper sludge and WASL applications enhanced Pb, Cd and Ni concentration in annual ryegrass and kale soils; however, these values were well below the Canadian Council of Ministers of the Environment (CCME) allowable limits for biosolid application. BC addition decreased 16% Pb concentration in annual ryegrass shoot whereas, Ni and As by 31% and 65%, in kale shoot. These results suggest that WA application has demonstrated agronomic benefits to both crops and thus could be used as an alternative source for liming and nutrients in the agriculture production systems. Paper sludge and WASL application exhibited lower heavy metal concentration in soil than the permissible limits for biosolids application. BC amendment in WA and SL treatments significantly reduced heavy metals uptake in both crops, hence could be a viable approach in reducing heavy metals uptake in forage and horticultural crops.

2.1 Introduction

Pulp and paper industries generate tremendous volume of waste products in North America (Cherian & Siddiqua, 2019). For instance, Canada produces more than 1M Mg of WA and 4.7 Mg of SL annually. In addition, SL is expected to increase from 48-86% in the next 50 years (Cherian & Siddiqua, 2019; Likon & Trebe, 2012). Managing such large volume of wastes could have major environmental and financial implications for the sector (Adiansyah et al., 2015). Sustainable management of this large volume of waste in an economically viable, socially acceptable, and environmentally sound manner is one of the most critical challenges faced by the paper industries across the globe (Cherian & Siddiqua, 2019; Likon & Trebe, 2012; Simão et al., 2018). Therefore, there is an urgent need to develop long-term sustainable management strategies to reduce environmental and financial burden that can accrue from these wastes byproducts (Tayebi-Khorami et al., 2019). In addition, it is necessary to develop sustainable management strategies for these wastes considering in recent years, disposal options such as landfill sites are being restricted, and in some regions or territories, it is prohibited by legislation (Cherian & Siddiqua, 2019). One sustainable management option is to repurpose WA and SL as a liming and nutrient sources that could not only minimize landfilling practice, but also be beneficial for improving soil physiochemical properties and plant growth (Hannam et al., 2018). Previous studies have shown that WA and SL increased soil pH due to the presence of carbonates and bicarbonates which neutralized hydrogen ion concentration in the soil solution (Royer-Tardif et al., 2019). An incubation study conducted by Yang et al. (2018) reported increase in pH of loamy sand soil by adding WA as neutralizing agent as compared to limestone, that might be due to smaller particle size and higher solubility of WA with water as compared to conventional limestone. Moreover, WA contain essential nutrients for plant growth, such as Ca, Mg, K and P and trace elements. For instance, in a three-year study, application of WA increased soil pH, Ca and Mg concentrations in sugar maple trees. WA as soil amendment increased growth diameter of mature sugar maple trees (Arseneau et al., 2021; da Costa et al., 2020; Pitman, 2006). It has been observed that WA application enhanced 94% biomass of *Brachiaria Brizantha* (Bonfim-Silva et al., 2017). Similarly, SL as a waste product is a rich source of organic matter, and plant essential nutrients such as N, P and Ca (Fahim et al., 2019). Ríos et al. (2012) reported that SL as soil amendment increased organic matter and P content in the soil, consequently increased the biomass of *Lolium perenne* L. Therefore, WA and SL amendment in acidic and shallow soils could be instrumental to increase soil pH, fertility status, thus plant growth and yield. In addition, paper mills consume a significant quantity of oil to burn timber for power generation. During the combustion process, trace elements (heavy metals) volatilize and condense forming WA (Johansen et al., 2021; Mortensen et al., 2018). Heavy metals (As, Cr, Cd, Pb and Ni) are non-biodegradable elements that affect plant metabolism and reduce crop yield. In addition, bioaccumulation of these heavy metals into fruits, vegetables and forages can cause adverse effects on animals and human health if the levels when consumed are more than the permissible CCME limits (Imeri et al., 2019; Khan et al., 2019). Due to these negative impacts, the presence of heavy metal in WA and SL can limit their use as agricultural soil amendments. However, organic soil amendments such as biochar (BC) could be a reliable source to retain heavy metals in the soil and reduce their uptake by plants, thus prevent heavy metals entering into the food chain (Shaaban et al., 2018).

Biochar is a black carbon or solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment (Xu et al., 2013). Studies in the literature report BC can enhance carbon sequestration, confer soil health benefits and can endure in soil for thousands of years (Zhang et al., 2013). Besides, BC has high pH, CEC, and ability to stabilize heavy metals in soils thereby limiting their uptake by crops (Zhang et al., 2013). The capacity of BC to adsorb heavy metals depends upon various factors such as pyrolysis process, residence time, temperature, type of feedstock and moisture content of feedstock (Liu et al., 2017).

Newfoundland soils are predominantly podzolic and cover 60% of the land mass in the province. Podzols are darker alluvial soils with low pH having high amounts of aluminum (Al) and iron (Fe) oxides as well as organic matter (Sanborn et al., 2011). Dissolved Al^{3+} in podzols is very toxic to plants. For example, at low pH, Al ions damage the root apex, limiting the uptake of essential nutrients which eventually results in poor growth and yield. In addition, podzolic soils contain clay minerals and iron oxides that fix phosphorous resulting in low plant uptake. (Kopittke et al., 2015; Truskavets'kyj et al., 2018; Yamamoto, 2019). Excessive aluminum and phosphorus unavailability are the main cause of the low soil fertility limiting agriculture production in podzolic soils (Zheng, 2010). Currently, commercial limestone is used as a liming source to raise the low soil pH in acidic soils such as podzol. This is not a cost-effective practice. Additionally, poor fertility status of NL soils, forced farmers to add high amount of expensive chemical fertilizers in an effort to enhance production, but this fertilizer input increases the cost of food production. WA and SL contain high pH and significant source of essential nutrients, which could be used as potential liming and nutrient sources for agriculture production in podzolic soils. Various studies have demonstrated WA, and SL as liming agent and nutrient sources in other jurisdictions. However, WA, SL alone and in combination with BC have not been evaluated as liming and nutrient sources in podzolic soils of NL. It was hypothesized that WA, SL and BC application will raise soil pH, improve crop growth, yield, N uptake and reduce heavy metals in plant tissues of ryegrass and kale. To test this hypothesis, a greenhouse experiment was conducted with the following specific objectives:

i. To investigate the effects of WA, SL alone and in combination with BC on seedling emergence, chlorophyll, photosynthesis and yield of annual ryegrass and kale.

ii. To examine the effects of WA, SL alone and in combination with BC on soil pH, N uptake and heavy metals uptake in annual ryegrass and kale.

2.2 Materials and methods

2.2.1 Study site

A greenhouse experiment was conducted at Provincial Tree Nursery, Grand Falls-Windsor, Canada during 2019. Soil was collected from newly cleared forest land, air dried, sieved with 4 mm sieve and thoroughly homogenized prior to analysis. A composite subsample was sent to the Soil, Plant and Feed Laboratory, Department of Fisheries, Forestry and Agriculture, St. John's, NL for detailed characterization. Detailed analysis was performed using the following methods. The pH of WA and SL were determined from saturated pastes. Buffer pH was analyzed on soils having a pH of 6.0 or less and was used to determine how much lime is required on farm soils (Hendershot et al., 1993) and EC was measured using a 2:1 (v/v) extract, solutions and analyzed with a EC meter at room temperature (Greenberg et al., 1992). Potassium, Mg, Ca and Na were determined using an ICP-OES following extraction with 1.0 N ammonium acetate solution (Simard, 1993). The concentration of P in the extract was determined colorimetrically using a continuous flow analyzer (Bran Luebbe AA3, Seal Analytical Inc., Mequon, WS) (Reid, 1998) following extraction with 0.5M Na_2CO_3 solution. Total C and N were measured by dry combustion using a Leco CN828. Heavy metals in WA and SL were determined by microwave-assisted acid digestion and ICP-MS analysis (USEPA-3052). Mineral N (NO_3^- and NH_4^+) were extracted using 2M KCl and analyzed by flow-injection (USEPA 353.2). Calcium carbonate equivalent (CCE) of WA and SL was determined by acid digestion using HCl (Black, 1965). The analysis results are shown in Table 2.1.

Table 2.1: Selected chemical properties of soil, wood ash (WA) and paper sludge (SL) used in current study

Parameters	Soil	Wood ash	Paper sludge	CCME limits for biosolids
pH	5.7	12.6	8.2	
EC	0.04	0.009	0.0003	
Organic matter	2.7			
Potassium, extractable	29.8	2420	1360	
Calcium, extractable	116	23600	5440	
Ammonium-NH_4^+	4.79	0.75	876	
Nitrate-NO_3^-	0.61	5.09	2	
Total Nitrogen	500	301	1040	
Organic carbon	1.29	3.45	39.9	
Arsenic	8.8	2.5	2.1	41
Cadmium	0.09	1.3	3.1	15
Chromium	36	130	55	1000
Cobalt	8.2	14	5.1	150
Copper	13	190	94	1500
Lead	7.9	20	35	300
Mercury	0.05	0.04	0.2	4
Molybdenum	0.42	16	6.3	20
Nickel	19	88	42	180
Selenium	0.36	0.17	0.44	25
Zinc	31	670	890	1850
CCE		10.4	12.9	

Note: All units are in $mgkg^{-1}$, dSm^{-1} for EC (Electrical Conductivity) and percent (%) for organic carbon, organic matter and CCE (Calcium Carbonate Equivalent). CCME = Canadian Council of Ministers of the Environment.

WA samples were air dried and sieved through 2mm sieve and mixed thoroughly to make a composite sample, prior to analysis. SL samples were air dried and grounded with Wiley Mill (Arthur H. Thomas). Thereafter, subsamples of WA and SL were sent to the Agriculture and Food Laboratory, University of Guelph for complete analysis, results are presented in Table 2.1. BC used in this study was analyzed by Gabilan laboratory, Salinas, California, USA, results are presented in Table 2.3.

2.2.2 Experimental treatments and design

Experimental treatments were Limestone (Control), WA, SL, WASL without BC addition (0 Mg ha^{-1}) and Control, WA, SL, WASL with addition of (20 Mg ha^{-1} BC). The greenhouse experiment was a randomized complete bock design with three replications. Annual ryegrass and kale were used as test crops. Limestone, WA, and SL were applied at 7.1, 17.25, and 55 Mg ha^{-1}, respectively. The WASL treatment was formulated as a mixture of WA and SL at 13.8 and 11 Mg ha^{-1}, respectively. Limestone, WA, SL, and WASL rates were calculated based on the calcium carbonate equivalent (CCE) and the lime requirement of the soil (Equation 1).

$$\text{Application rate} = \frac{\text{Area} \times \text{Lime requirement (Mg/ha)}}{\text{CCE} \times (100 - \%\ \text{Moisture content})} \qquad (1)$$

Different rates (100%, 50% and 25%) of the amendments were tested with a pH meter to determine the optimum rate that could attain the target pH (6.3) (Table 2.2).

Table 2.2: pH results obtained from application of soil amendments with different rates on weight basis

Wood ash rate (Mgha^{-1})	pH	Paper sludge rate (Mg ha^{-1})	pH
69 (100%)	7.05	78 (100%)	6.6
34.5 (50%)	6.7	55 (70 %)	6.3
17.25 (25%)	6.3	27.5 (25 %)	5.9

Table 2.3: Physicochemical properties of biochar used in this study

Properties	Dry weight basis
pH	9
EC (dS/m)	0.43
Moisture (%)	-
WHC (mL water per 100 g dry char)	74.9
Volatile matter (%)	8.5
Ash (%)	6.7
Fixed carbon (%)	84.5
H (%)	0.68
O (%)	7.84
N (%)	0.22
S (%)	0
H/C	0.1
O/C	0.07
Total ash (%)	7.1
Recalcitrant carbon (%)	76.2
Neutralizing value (% as $CaCO_3$)	4.9
Carbonate value (% as $CaCO_3$)	0.6
Bulk density (Mg/m^3)	0.19
Particle density (acetone)(g/cc)	1.57
Solid space (%v/v)	12.5
Void space (% v/v)	87.5

Calculated amounts of amendments for each treatment were thoroughly mixed with air-dried soil. Pots were filled with the amended soil 2 kg for ryegrass and 5 kg for kale and added to respective pots. Each pot was watered to 60% water filled pore space (WFPS) with tap water and left for 7 days for soil stabilization before seeding. Step by step procedure of experimental setup have shown in th Figure 2.1.

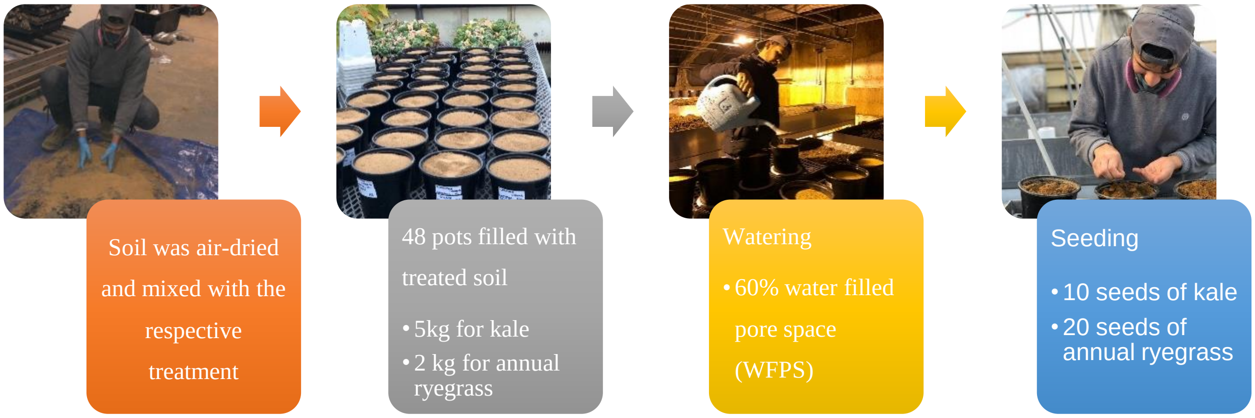

Figure 2.1: Step by step procedure; left to right, drying of soil, pots filling with soil and amendments, watering and seeding of annual ryegrass and kale.

After seven days, 10 seeds of kale and 20 seeds of annual ryegrass were directly seeded into the pots at 1 cm depth (Ashenafi & Tewodros, 2018; Park et al., 2012). After correcting for mineral N and total P and K in amendments, annual ryegrass and kale pots were fertilized with urea, triple super phosphate and muriate of potash to supply 150:87:149 kg NPK, and 180:52:128 kg NPK per/ha, respectively. Throughout the study, greenhouse temperature was maintained at 23 ± 2 °C for 16 h during the day and 15 ± 1 °C for 8 h at night and at 65 ± 5 % average humidity (Vidal et al., 2018).

2.2.3 Crop growth and yield measurements

Seedling emergence number was recorded daily for 14 days. At 14 days after sowing (DAS), plants were thinned to 16 plants per pot for annual ryegrass and one plant per pot for kale. The plants were nurtured for 55 DAS for annual ryegrass and 90 DAS before harvesting. At harvest, plant height, chlorophyll, photosynthesis rate and yield were measured. Plant height of annual ryegrass and kale was measured with a measuring tape from the base of the plant to the highest growing point (Merkl et al., 2004). Chlorophyll of annual ryegrass and kale was measured using a handheld chlorophyll meter (SPAD 502 Plus chlorophyll meter, Minolta, Japan). Photosynthesis rate was measured by a portable photosynthesis system (LI-6400XT, Licor Biosciences, Lincoln, USA). Before photosynthesis measurements, the photosynthesis system was configured at 400 μL L^{-1} CO_2 concentration, 25 °C air temperature, and 1000 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). After growth parameter measurement, annual ryegrass plants were harvested at early ear emergence stage (55 DAS) (Celis-Alvarez et al., 2016) and kale plants were harvested at full physiological maturity (90 DAS) (Karim et al., 2015), respectively and their fresh weights were recorded. Plant shoot samples were then oven dried at 65 °C for 72 h and dry weights were measured to reflect the dry matter yield (DMY) (Solomon et al., 2017). Oven dried plant samples were pulverized using a ball-mill grinder (Retsch Cryomill, Hann, Germany) and thoroughly homogenized before analyses.

2.2.4 Soil sampling

After harvest, the soil was taken out from each pot, thoroughly mixed and a 500 g sub sample was collected from each treatment for laboratory analyses. A 50 g of moist soil from each collected sample was sieved with a 2 mm sieve size and stored at 4 °C for residual N analysis (NH_4^+ and NO_3^-) following the method of Ashiq et al. (2020). The rest of the soil was air dried, sieved with a 2 mm mesh and ground using a mortar and pestle prior to analysis for total carbon, total nitrogen and heavy metal concentrations (Bertsch & Ostinelli, 2019; Hagemann et al., 2017).

2.2.5 Laboratory analyses

2.2.5.1 Residual nitrogen

Residual N (NH_4^+ + NO_3^-) in soil samples after crop harvest was determined following the method reported by Hagemann et al. (2017). Briefly, 50 mL of 2*M* KCl was added to 5 g moist soil in a 125 mL Erlenmeyer flask. The soil-KCl suspension was shaken for 30 min on a benchtop open air platform shaker (New Brunswick™ Innova® 2300, Eppendorf Canada, Mississauga, Ontario). A 10 g subsample was oven dried at 105 °C for 24 h to determine moisture content (MC) (Equation 2.1).

After shaking, flasks were left at room temperature for 30 min for soil settling (Hagemann et al., 2017) and the solution was filtered into 20 mL scintillation vials. Filtered samples were stored at -20 °C until time of analysis. NH_4^+ and nitrate (NO_3^-) in soil extracts were analyzed using Auto analyzer (Seal analytical continuous flow analyzer (AA3 HR) (Hagemann et al., 2017). NO_3^- present in extracts was reduced to nitrite (NO_2^-) by cadmium-copper reduction column at pH 8, NO_2^- formed in this process then reacts with sulfanilamide to form a diazo compound. Resulted compound then reacts with N-1-naphthylethylenediamine dihydrochloride to form a reddishpurple diazo dye. NH_4^+ was determined by using salicylate method and the final calculations were done using following formulas (Eq. 2.1, Eq. 2.2 and Eq. 2.3).

$$\text{Moisture content} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \qquad \text{(Eq. 2.1)}$$

Soil NH_4^+ and NO_3^- was calculated in moist soil by:

$$NH_4^+ \text{ and } NO_3^- \text{ (mg g}^{-1}\text{ of wet soil)} = NH_4^+ \text{ and } NO_3^- \text{ in soil extract (mg L}^{-1}\text{) x 10} \qquad \text{(Eq. 2.2)}$$

NH_4^+ and NO_3^- were multiplied by 10 as soil-solution ratio was 1: 10 in soil extracts.

Soil NH_4^+ and NO_3^- in dry soil was calculated by the following formula:

$$NH_4^+ \text{ and } NO_3^- \text{ (mg g}^{-1}\text{ of dry soil)} = NH_4^+ \text{ and } NO_3^- \text{ in moist soil x MC} \qquad \text{(Eq. 2.3)}$$

2.2.5.2 Soil pH

Soil pH was determined following the method of Park et al. (2012). A 10 g air dried soil was taken from the bulk soil sample collected after harvest of both crops and added into 50 mL sterile plastic tubes and 20 mL of deionized water was added into each tube (1 soil:2 water). All tubes were shaken for 30 min and kept them stand vertically for 1 h. The pH was measured using a handheld pH meter (Bluelab Combo Meter, Tauranga, New Zealand).

2.2.5.3 Heavy metals in soil and plant tissues

Heavy metals in plant tissues were determined following the method of Ali et al. (2019). Briefly, a 100 mg of plant sample from each crop was mixed with 10 mL concentrated (67% - 70 %) nitric acid (HNO_3) in digestion tubes. Each digestion tube was tightly caped, and samples were digested using a microwave digestion system (Multiwave Go Microwave Digestion System; Anton Paar, United States) for 20 min to completely digest the samples. The digestion tubes were cooled at room temperature, and samples were filtered into 50 mL sterile plastic tubes and stored at 4 °C. Before analysis, samples were diluted with deionized distilled water. Heavy metal concentrations in extracts were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Thermo Scientific iCAP Q ICP-MS). Pure ICP-MS standard solution (IV–ICPMS–71A) with multi elements (43) purchased from Inorganic™ Ventures, Inc. (Christiansburg, VA 24073, USA) was used to calibrate instrument range from 0.971 to 1.000. Soil samples were digested following the same procedure as mentioned above for plant tissues and then analyzed for heavy metal concentration using the same ICP-MS.

2.2.5.4 Total carbon (C) and total nitrogen (N)

Total C and N in soil samples were measured following the method reported by Bertsch and Ostinelli (2019). Briefly, a 2 g of air dried and ground soil sample from each soil was weighed into a ceramic boat and analyzed for C and N by dry combustion method using LECO TruSpec CN autoanalyzer (LECO Corporation, St. Joseph, MI, USA) (Partey et al., 2014). Samples were introduced into a furnace at 950 °C, CO_2 and N gas formed from the combustion process mixed with pure oxygen and passed through an infrared (IR) detector that measured the IR energy. Calculated IR energy is directly proportional to C concentration in the sample whereas total N concentration was determined by passing N gas through a thermal conductivity detector. C/N ratio was calculated by dividing total C to total N concentration in the sample (Wiater, 2020).

2.2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effect of soil amendments and BC treatments on growth, yield, residual nitrogen concentration and heavy metals in shoots and soil of annual ryegrass and kale. Normality of the data set was checked by running Shapiro-Wilkes test. Means of treatment were compared with Fisher's least significant difference (LSD) test ($\alpha = 0.05$). The data were analyzed using the Statistix 10 software package (Analytical software, FL, USA) and graphs were prepared by Origin Pro, 2021 software program (OriginLab Corporation, Northampton, MA, USA). To check the overall association between soil amendments, plant growth and heavy metal dynamics, redundancy analysis (RDA) was performed using XLSTAT software (Premium 2017, Version 19.5; Addinsoft, Paris, France). Pearson's correlation analysis was done to determine the strength of the relationships among annual ryegrass and kale with observed parameters.

2.3 Results

2.3.1 Growth parameters

2.3.1.1 Annual Ryegrass

Final emergence and plant height in annual ryegrass were unremarkable regardless of treatments and BC application (Table 2.4). Emergence ranged between 80 and 91 %. Across all treatments, the average plant height was 51.5 cm. Chlorophyll did not vary by amendment type but was significantly affected by BC application. The addition of BC lowered chlorophyll by 10.2 %. There was a significant amendment × BC interaction on photosynthesis rate (Figure 2.2). Although among amendments (WA, SL and WASL), addition of BC did not cause any significant effect on photosynthesis rate. However, when BC was added to control, photosynthesis values decreased by 30% relative to control without BC. Furthermore, photosynthesis in WA was significantly lower than the control, whereas SL and WASL did not differ from WA or control. When BC was added, there were no significant differences among amendments.

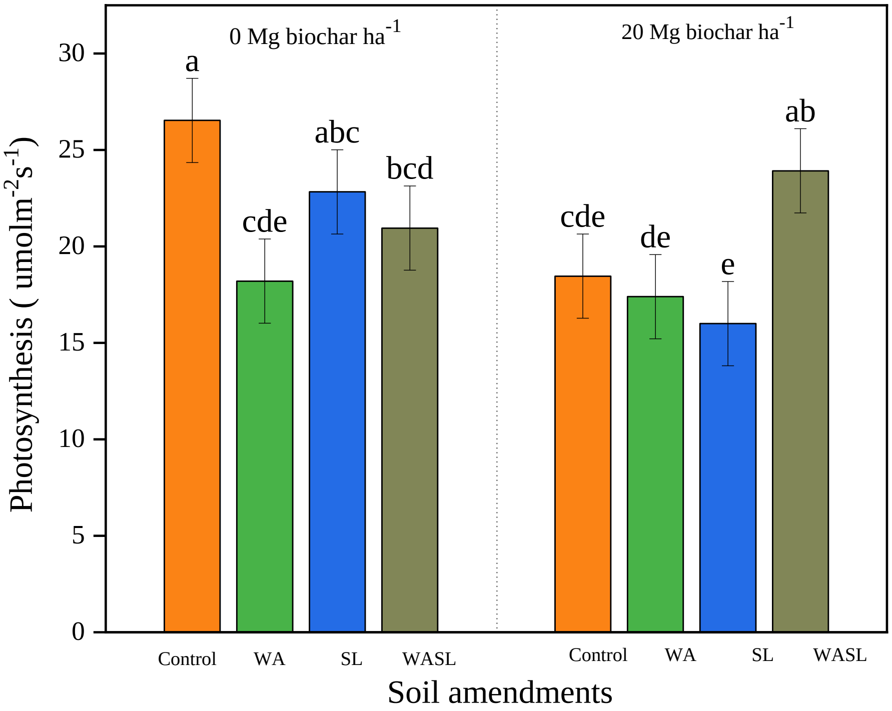

Figure 2.2: Effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on photosynthesis rate in annual ryegrass under controlled environment conditions. Values in bar chart (μmol m^{-2} s^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different (*P* < 0.05), Fisher's LSD test.

2.3.1.2 Kale

There were no significant effects of amendment or BC on kale emergence, chlorophyll or number of leaves (Table 2.5). On average, emergence ranged between 88 and 94 %. The highest plant height (40.9 cm) was observed in WA whereas the lowest was recorded in WASL (34 cm).

2.3.2 Biomass yield

2.3.2.1 Annual Ryegrass

Soil amendments had a significant effect ($P < 0.01$) on yield of ryegrass, whereas BC did not significantly influenced yield of ryegrass (Table 2.4). WA produced 71% greater yield (1.88 Mg ha^{-1}) compared to the control (1.10 Mg ha^{-1}) (Table 2.4), whereas SL and WASL did not significantly differ from WA and control.

Table 2.4: Means and analysis of variance (ANOVA) showing the effects of soil amendments, biochar and their interaction on soil pH, plant growth, yield, residual nitrogen, C/N ratio and nitrogen uptake in annual ryegrass under controlled environmental conditions (3 replications and n = 24).

Source of variation	Seedling Emergence %	Plant height (cm)	Chlorophyll (SPAD values)	Photosynthesis rate (µmolm^{-2}s^{-1})	Yield (Mg ha^{-1})	N uptake (kg ha^{-1})	Residual N (mg kg^{-1})	C/N ratio
Amendments (AM)								
Control	87	50.7	47.1	22.4 a	1.10 c	40.3 c	43.5 a	26.0
WA	91	53.1	48.3	17.7 b	1.88 a	59.9 a	21.8 c	26.3
SL	91	50.1	46.7	19.4 ab	1.42 b	40.4 c	27.7 b	28.8
WASL	80	52.3	46.8	24.4 a	1.62 b	51.1 b	12.4 d	27.5
BC (Mg ha^{-1})								
0	88	51.8	49.8 a	22.1 a	1.52	49.8	28.3 a	24.2 b
20	86	52.3	44.6 b	18.9 b	1.49	46.1	24.4 b	30.0 a
				P - value				
AM	0.08	0.33	0.74	0.02	< 0.01	< 0.01	< 0.01	0.12
BC	0.51	0.69	< 0.01	< 0.01	0.06	0.19	< 0.01	< 0.01
AM × BC	0.62	0.90	0.06	0.02	0.28	0.17	< 0.01	0.21

Table 2.5: Means and analysis of variance (ANOVA) showing the effects of soil amendments, biochar and their interaction on soil pH, plant growth, yield, residual nitrogen, C/N ratio and nitrogen uptake in kale under controlled environmental conditions (3 replications and n = 24).

Source of variation	Emergence %	Plant height (cm)	Number of leaves (plant^{-1})	Chlorophyll (SPAD values)	Yield (g plant^{-1})	N uptake (mg pot^{-1})	Residual N (mg kg^{-1})	C/N ratio
Amendments (AM)								
Control	93	36.7 ab	12.3	46.0	79.8 b	630 a	3.44 b	23.5 b
WA	91	40.9 a	13.1	44.9	89.3 a	659 a	2.71 c	24.6 b
SL	90	35.2 b	13.8	42.8	77.9 b	457 b	1.40 d	26.8 a
WASL	92	34.1 b	1.8	45.9	84.2 ab	537 b	4.26 a	25.0 b
BC (Mg ha^{-1})								
0	88	36.6	13.5	45.1	85.7 b	572	2.81	23.7 b
20	94	36.8	13.0	44.8	79.9 a	569	3.09	26.3 a
P - value								
AM	0.94	0.03	0.09	0.12	0.01	< 0.01	< 0.01	< 0.01
BC	0.06	0.88	0.20	0.80	0.01	0.94	0.09	< 0.01
AM x BC	0.16	0.58	0.93	0.70	0.01	0.95	< 0.01	0.14

2.3.2.2 Kale

There was a significant × BC effect on kale yield (Table 2.5). Higher kale yield (96 g plant^{-1}) was observed in WA without BC amendment compared to the lowest (73 g plant^{-1}) recorded in SL amended with BC (Figure 2.). BC addition in amendments did not affect yield when compared with non-amended BC treatments. However, WA without BC addition showed 28 % more yield than non-amended BC Control (Figure 2.).

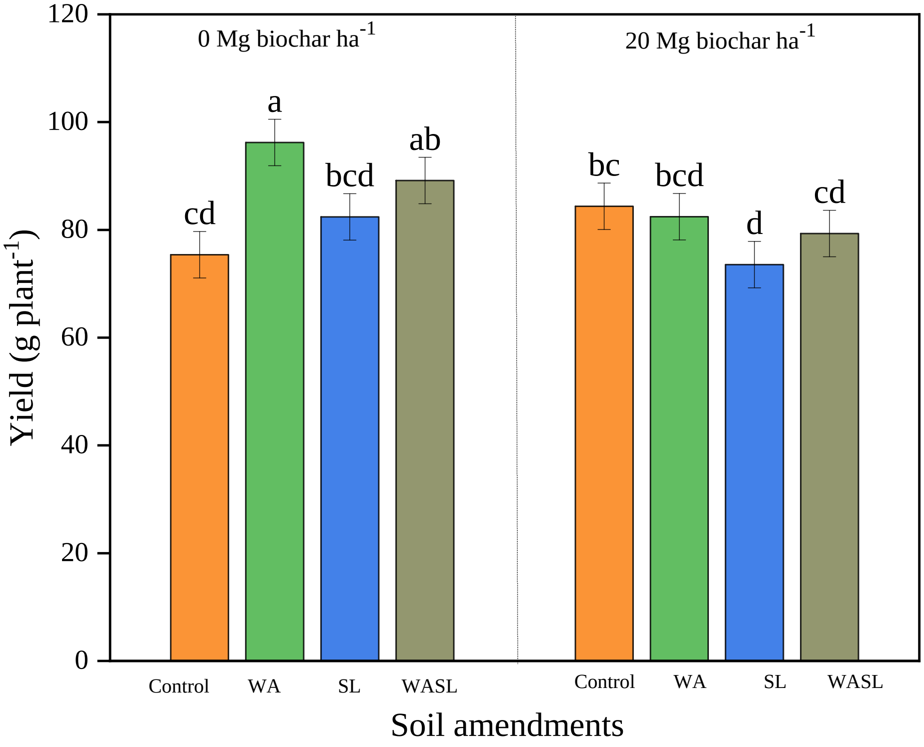

Figure 2.3: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on kale yield under controlled environmental conditions. Values in bar chart (g plant^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($P < 0.05$), Fisher's LSD test.

2.3.3 Nitrogen uptake

2.3.3.1 Annual Ryegrass

Soil amendments showed significant ($P = 0.02$) effects on N uptake (Table 2.4). WA showed highest N uptake (60 kg ha^{-1}), though statistically at par with non-amended WASL treatment and lowest (40 kg ha^{-1}) uptake was observed in control (Table 2.4). Annual ryegrass plants showed 33 % more uptake when treated with WA compared to control (Table 2.4).

2.3.3.2 Kale

In kale, soil amendments showed significant effects ($P < 0.001$) on N uptake contrary to non-significant effects of BC application rates and interactive effects of BC and soil amendments (Table 2.5). Higher N uptake values (659 mg pot^{-1}) was observed in WA which was statistically not different from control whereas the lowest N uptake (457 mg pot^{-1}) was observed in SL treatment (Table 2.5).

2.3.4 Residual nitrogen

2.3.4.1 Annual Ryegrass

In annual ryegrass AM × BC had significant effects on residual N (Table 2.4). Non-amended BC control showed higher (44 mg kg^{-1}) residual N concentration compared to lowest (13 mg kg^{-1}) in WASL without BC application. Among non-amended BC treatments, WA and WASL showed 72 and 84 % lower residual N, compared to non-amended BC control. Similarly, BC amended WA, SL and WASL exhibited 89, 91 and 91 % less residual N than BC amended control. Residual N concentration in BC amended WA significantly increased by 40 % compared to WA without BC application. Whereas SL amended with BC displayed lower concentration of residual N by 69 % than non- amended SL with BC (Figure 2.).

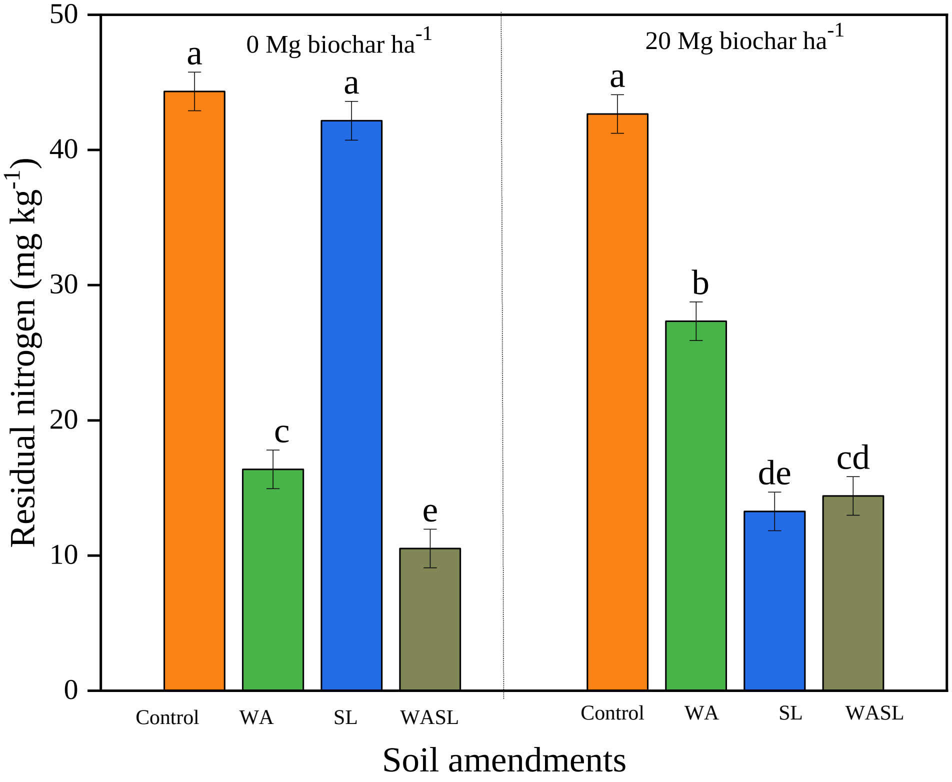

Figure 2.4: Effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on annual ryegrass residual nitrogen in soil under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($P < 0.05$), Fisher's LSD test.

2.3.4.2 Kale

There was a significant ($P < 0.001$) interaction of AM and BC (Table 2.5). Highest (4 mg kg^{-1}) residual N was observed in control without BC addition, though statistically similar with WASL without BC application and BC amended WA and WASL treatments. Lowest (1.13 $mgkg^{-1}$) residual N concentration was observed in WA without BC addition. BC addition significantly increased residual N in WA by 28 % compared to non-amended WA with BC (Figure 2.). BC amended control exhibited 48 % lower residual nitrogen than non- amended BC control, whereas BC amended WA enhanced residual nitrogen in soil compared to non-amended BC WA by 79 %. Additionally, among non-amended BC treatments WA and SL showed lower residual nitrogen than control, whereas BC addition increased residual nitrogen concentration compared to control amended with BC.

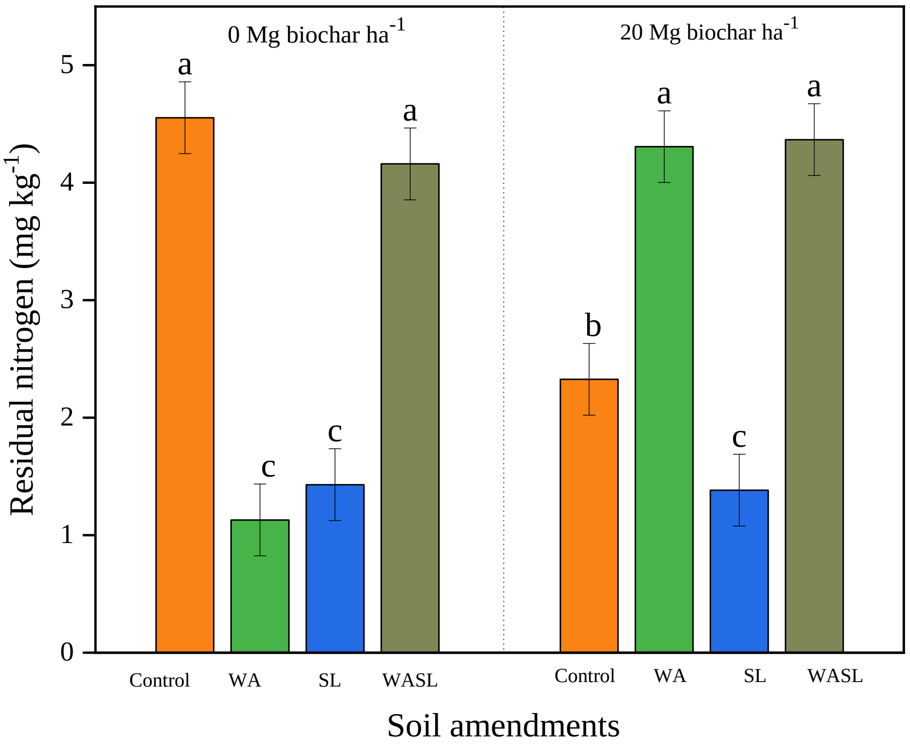

Figure 2.5: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on kale residual nitrogen in soil under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher’s LSD test.

2.3.5 C/N ratio

2.3.5.1 Annual Ryegrass

BC application significantly ($P < 0.001$) affected the C/N ratio of annual ryegrass soil (Table 2.4). Higher C/N ratio was observed in BC amended treatment compared to lowest in non-amended BC treatment in ryegrass soil. BC addition showed 20 % higher C/N ratio compared to non-BC amended soil (Table 2.4).

2.3.5.2 Kale

Amendments and BC application significantly ($P = 0.001$, $P < 0.001$) affected C/N ratio in kale soil (Table 2.5). Higher C/N ratio (27) was observed in SL, though statistically non-significant with WA and WASL treatments. Lower C/N ratio (23) was recorded in control. BC amended soil exhibited 13% higher C/N ratio than non-amended BC treatment (Table 2.5).

2.3.6 Soil pH

2.3.6.1 Annual Ryegrass

Amendments significantly ($P < 0.001$) affected soil pH during cultivation of annual ryegrass (Table 2.6). Soil pH in WA and WASL was not different from that in control. However, pH in SL was 13%, 6%, and 6% lower than WA, WASL and control respectively (Table 2.6).

2.3.6.2 Kale

Similarly, amendments had a significant ($P < 0.001$) effect on soil pH of kale (Table 2.8). Soil pH in WA and WASL was not different when compared with control, whereas SL showed 6 % lower pH than WA and WASL, respectively (Table 2.8).

2.3.7 Heavy metals in plant tissues and soil

2.3.7.1 Annual Ryegrass

Soil amendments, and BC application had significant effects ($P = 0.02$) on Cr concentration in the soil following cultivation of annual ryegrass. A higher Cr concentration (28 mg kg^{-1}) was observed in WA which was statistically at par with control, and the lowest Cr concentration (24 mg kg^{-1}) was recorded in WASL amended soil (Table 2.6). BC amended soil exhibited 7 % higher Cr concentration than non-amended BC soil (Table 2.6). Whereas soil amendments BC and their interaction had non-significant effects on Cr concentration in shoot tissues (Table 2.7).

Soil amendment, BC and their interaction showed nonsignificant effects on Ni concentration in soil and shoots (Table 2.6 Table 2.7).

Soil amendments and BC had significant effects ($P < 0.001$ & $P < 0.001$) on Cu concentration in annual ryegrass soil (Table 2.6). Highest (8.9 mg kg^{-1}) Cu concentration was observed in WA compared to lowest (6.9 mg kg^{-1}) Cu concentration in control. BC amended soil showed 13 % higher Cu concentration than non-amended BC soil (Table 2.6). Similarly, soil amendments and BC application had significant effects ($P < 0.001$, $P < 0.001$) on Cu uptake in shoot tissues (Table 2.7). A higher (2.8 mg kg^{-1}) Cu uptake in ryegrass shoot tissues was observed in SL, though statistically non-significant with WA treatment. WASL treatment exhibited lower Cu concentration (0.58 mg kg^{-1}) in ryegrass shoot tissues compared to control and other treatments (Table 2.7). BC amended soil exhibited significantly lower Cu uptake than non-amended BC in shoots (Table 2.7).

BC application had significant ($P = 0.03$) effects on As concentration in soil (Table 2.6). BC application significantly increased As concentration by 10 % compared to non-amended BC treatment (Table 2.6). However, soil amendments, BC and their interaction had no significant effects on the As uptake in shoot tissues (Table 2.7).

Soil amendment and BC application had significant ($P < 0.001$ & $P = 0.02$) effects on lead (Pb) concentration in soil (Table 2.6). Higher Pb concentration was noted in SL (6.8 mg kg^{-1}) and lowest (6 mg kg^{-1}) was observed in WA treatment. BC amended treatment exhibited 5 % higher Pb concentration than non-amended BC in annual ryegrass soil (Table 2.6). However, BC amended treatments had significant ($P = 0.02$) effects on Pb uptake in annual ryegrass tissues (Table 2.7). Soil amended with BC reduced 20 % Pb uptake than non-amended BC treatments in annual ryegrass shoots (Table 2.7).

Soil amendments and BC had significant effects ($P < 0.001$ & $P = 0.03$) on Cd concentration in soil (Table 2.6). Higher (0.12 mgkg-1) Cd concentration was observed in SL treatment whereas, lowest (0.03 mgkg-1) was recorded in control. BC addition enhanced Pb concentration in annual ryegrass soil by 25 % compared to non-amended BC soil (Table 2.6). However, soil amendments, BC application and their interaction had not significant effects on Cd uptake in annual ryegrass shoot tissues (Table 2.7).

Table 2.6: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar application on soil pH and heavy metal concentration (mg kg^{-1}) in soil of annual ryegrass crop under controlled environmental conditions (3 replications and n = 24).

Source of variation	Soil pH	Cr	Ni	Cu	As	Pb	Cd
Amendment (AM)							
Control	6.0 b	28 a	12	6.9 b	4.9	6.1 c	0.03 c
WA	6.2 a	28 a	13	8.9 a	4.8	6.0 c	0.05 bc
SL	5.6 c	26 ab	12	8.7 a	4.6	6.8 a	0.12 a
WASL	6.0 ab	24 b	12	8.6 a	5.2	6.4 b	0.06 b
Biochar (BC) Mg ha^{-1}							
0	5.9	25 b	12	7.7 b	4.6 b	6.2 b	0.06 b
20	5.9	27 a	13	8.9 a	5.1 a	6.5 a	0.08 a
				P - value			
AM	< 0.01	0.02	0.78	< 0.01	0.18	<0.01	< 0.01
BC	1.00	0.02	0.17	< 0.01	0.03	0.02	0.03
AM x BC	0.92	0.35	0.10	0.19	0.55	0.37	0.66

Table 2.7: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar application on soil pH and heavy metal concentration (mg kg^{-1}) in annual ryegrass shoots under Controlled environmental conditions (3 replications and n = 24).

Source of variation	Cr	Ni	Cu	As	Pb	Cd
Amendment (AM)						
Control	2.0	2.3	1.0 b	0.9	0.5	0.02
WA	2.8	2.4	2.5 a	0.3	0.6	0.05
SL	2.2	1.8	2.8 a	0.5	0.6	0.06
WASL	2.4	1.7	0.5 b	0.4	0.4	0.06
Biochar (BC) Mg ha^{-1}						
0	2.6	2.1	2.1 a	0.7	0.6 a	0.11
20	2.1	1.9	1.2 b	0.3	0.5 b	0.03
			P - value			
AM	0.41	0.23	< 0.01	0.61	0.10	0.43
BC	0.17	0.47	< 0.01	0.19	0.02	0.21
AM x BC	0.89	0.82	0.91	0.52	0.14	0.47

2.3.7.2 Kale

Soil amendments, BC, and their interaction had non-significant effects on Cr concentration in soil (Table 2.8). Contrarily, soil amendments and biochar interaction significantly affected Cr concentration in shoot tissues (Table 2.9). WASL without BC application exhibited higher Cr (1.9 mg kg^{-1}) uptake followed by lowest (0.65 mg kg^{-1}) in BC amended control. BC amended treatments significantly reduced Cr uptake in kale tissues grown in non-amended treatments (Table 2.9).

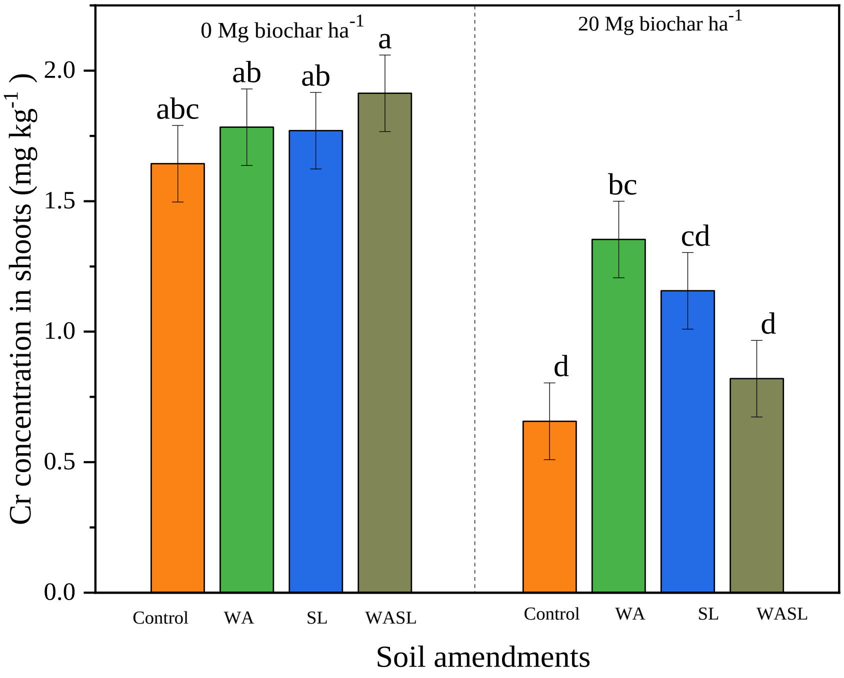

Figure 2.6: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on chromium concentration in kale shoots under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

Soil amendments and BC application rate had significant effects on ($P < 0.01$ & $P < 0.01$) Ni concentration in soil (Table 2.8). WASL amended treatment showed higher (15 mg kg^{-1}) Ni concentration compared to lowest (12 mg kg^{-1}) in control (Table 2.8). Soil amended with BC retained 13 % higher Ni as compared to non-amended BC soil (Table 2.8).

Application of BC had significant effects ($p < 0.01$) on Ni uptake in shoot tissues (Table 2.9). BC amended treatment showed significant reduction in Ni uptake (1.3 mg kg^{-1}) in shoot tissues of kale than non-amended BC treatment (1.9 mg kg^{-1}) (Table 2.9).

Soil amendments and BC application had significant effects ($P = 0.01$ & $P < 0.01$) on Cu concentration in soil (Table 2.8). Maximum Cu concentration (11 mg kg^{-1}) was noted in WA though statistically similar with SL and WASL treatments, whereas lowest (9 mg kg^{-1}) Cu concentration was observed in Control (Table 2.8). BC amended soil showed higher Cu concentration compared to non-amended BC soil (Table 2.8). Whereas significant interactive effect ($P = 0.02$) of soil amendments and BC application was observed in Cu uptake in shoot tissues (Figure 2.9). Non-amended BC treatments exhibited higher Cu uptake in kale shoot tissues whereas, BC amended treatments showed significant reduction in Cu uptake in shoot tissues. WA without BC addition exhibited higher (2.7 mg kg^{-1}) Cu uptake and statistically non-significant from Control whereas minimum (0.94 mg kg^{-1}) Cu concentration was observed in SL amended with BC (Figure 2.). BC amended WA, SL, WASL and control reduced 48, 39, 47 and 53% Cu uptake in the non-amended BC treatments.

Table 2.8: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar on soil pH and heavy metal concentration (mg kg^{-1}) in soil of kale crop under controlled environmental conditions (3 replications and n = 24).

Source of variation	Soil pH	Cr	Ni	Cu	As	Pb	Cd
Amendment (AM)							
Control	6.0 a	28	12 b	9.4 b	5.3 ab	5.7	0.07 b
WA	6.2 a	27	13 b	10 a	5.0 b	5.6	0.1 b
SL	5.8 b	28	14 a	11 a	5.3 b	5.9	0.2a
WASL	6.2 a	30	15 a	10 ab	6.0 a	5.8	0.2 a
Biochar (BC) Mg ha^{-1}							
0	6.0	27	13 b	9 b	5.0 b	5.6 b	0.1 b
20	6.1	29	15 a	11 a	6.0 a	5.9 a	0.2 a
				P - value			
AM	<0.01	0.35	<0.01	0.01	0.04	0.28	<0.01
BC	0.46	0.06	<0.01	<0.01	<0.01	0.01	0.01
AM x BC	0.89	0.86	0.89	0.28	0.64	0.33	0.25

Table 2.9: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar on heavy metal concentration (mg kg^{-1}) in shoots of kale crop under controlled environmental conditions (3 replications and n = 24).

Source of variation	Cr	Ni	Cu	As	Pb	Cd
Amendment (AM)						
Control	1.1	1.6	1.8	0.7	0.5	0.03
WA	1.6	1.4	2.1	0.5	0.4	0.02
SL	1.5	1.6	1.2	0.4	0.4	0.03
WASL	1.3	1.8	1.3	0.4	0.4	0.04
Biochar (BC) Mg ha^{-1}						
0	1.7	1.9 a	2.1	0.6	0.5	0.04 a
20	0.9	1.3 b	1.1	0.4	0.4	0.02 b
			P - value			
AM	<0.01	0.30	<0.01	0.13	0.03	0.42
BC	<0.01	<0.01	<0.01	0.08	<0.01	<0.01
AM x BC	0.02	0.29	0.02	0.16	0.03	0.57

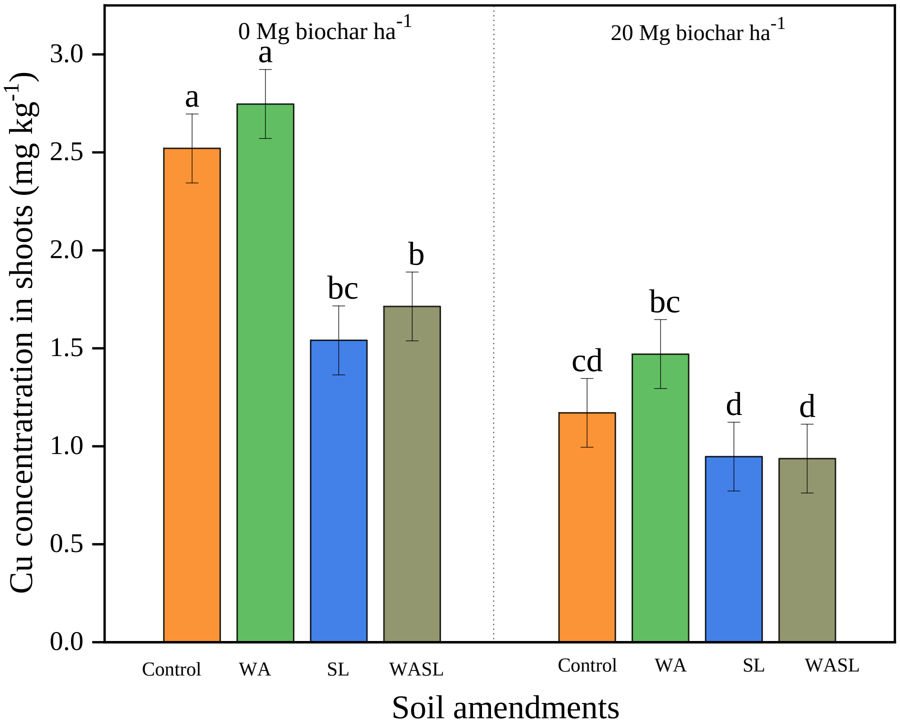

Figure 2.7: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on copper concentration uptake in kale shoots under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

Soil amendment and BC application had significantly ($P = 0.04$ & $P < 0.01$) affected As concentration in kale soil (Table 2.8). Maximum As concentration (5.8 mg kg^{-1}) was observed in WASL and minimum (5 mg kg^{-1}) was recorded in WA. BC addition increased As concentration in kale soil (Table 2.8). Contrarily, soil amendments, BC and their interaction had non-significant effects on As concentration in kale shoot tissues (Table 2.9).

BC addition had significant (*P* = 0.01) effects on Pb concentration in kale soil (Table 2.8). BC amended treatment exhibited higher concentration of lead than non-amended BC treatment (Table 2.8). Whereas soil amendments, and BC interaction had significantly affected (p = 0.03) Pb uptake in kale shoot tissues (Table 2.9). Non-amended BC control showed higher (0.55 mg kg^{-1}) Pb uptake whereas BC addition significantly reduced Pb uptake in kale shoot tissues, however, minimum was recorded (0.38 mg kg^{-1}) in SL amended with BC although statistically non-significant with all treatments except non-amended BC Control (Figure 2.8).

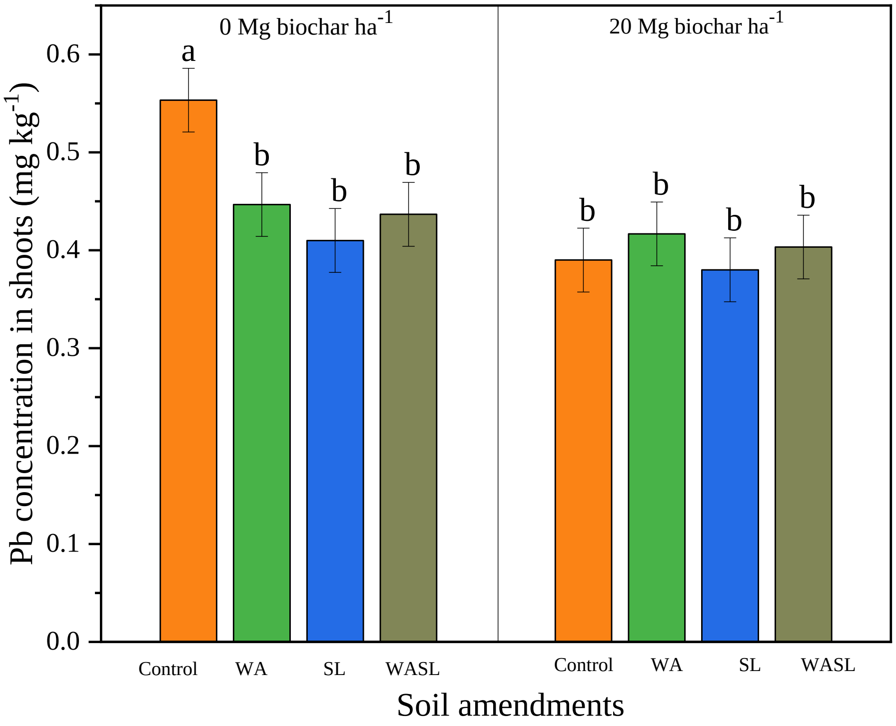

Figure 2.8: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on lead concentration in kale shoots under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Different letters indicate significant differences between treatments at p

< 0.05 level. Values showing the same letters are not significantly different ($P < 0.05$), Fisher's LSD test.

Soil amendments and BC application had significant ($P < 0.01$ & $P = 0.01$) effects on Cd concentration in soil (Table 2.8). Maximum Cd concentration (0.2 mg kg^{-1}) was observed in WASL compared to lowest (0.07 mg kg^{-1}) was noted in control treatment (Table 2.8). BC addition increased Cd concentration in soil compared to non-amended BC treatment (Table 2.8). Similarly, BC addition had significant effects ($P < 0.01$) on Cd uptake in shoot tissues of kale (Table 2.9). BC addition reduced 25% Cd uptake in shoot tissues compared to non-amended BC treatment (Table 2.9).

2.3.8 Relationship between soil amendments, agronomic performance and crop quality

2.3.8.1 Annual ryegrass

The plant height, chlorophyll, photosynthesis, nitrogen uptake and yield were used as indicator of agronomic performance, while heavy metals (Cr, Ni, Cu, As, Pb and Cd) were used as indicator of crop and soil quality. The output from RDA analysis revealed that axis 1 and axis 2 explained 64.06% and 20.05% of the total variance (Figure 2.9). RDA bi plot showed segregation of soil amendments into different quadrants representing three distinct groups clustering paper mill waste and two groups for BC treatments (Figure 2.9). The RDA biplot depicted relationship of different observed variables affected by soil amendments (Figure 2.9). RDA map expressed grouping of WA, WA+SL and BC treatments due to observed variables including N uptake, yield, plant height, soil pH (Figure 2.9). Whereas soils treated with BC 20 retained heavy metals and expressed lower heavy metal uptakes in growing plants (Figure 2.9). Overall, the RDA results indicate WA alone and in combination with SL (WA+SL) increased annual ryegrass growth, nitrogen uptake and yield whereas soil amendments along with biochar application reduced the heavy metal uptake in annual ryegrass shoots (Figure 2.9).

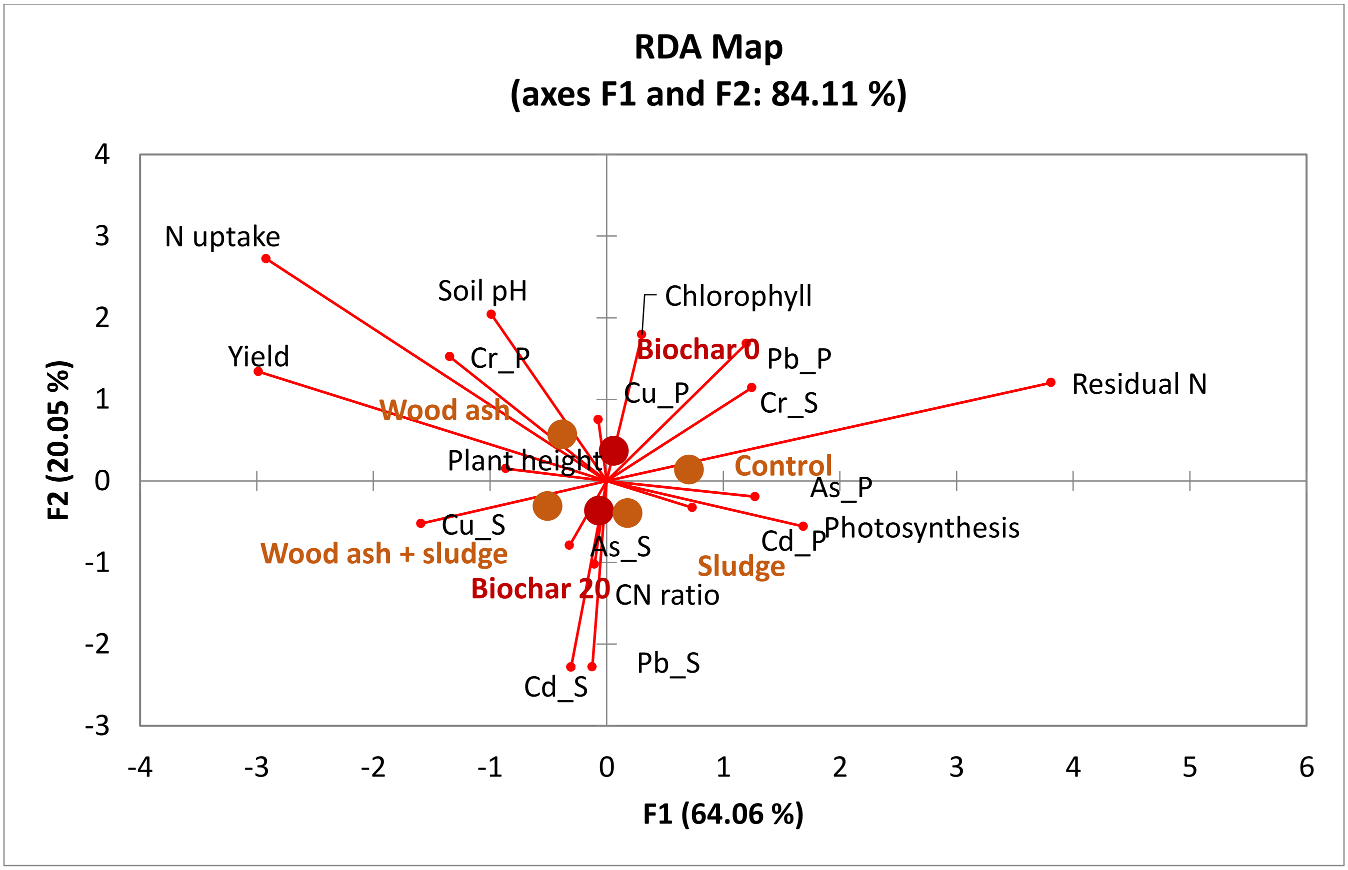

Figure 2.9: Redundancy analysis (RDA) of the growth parameters, heavy metals in soil, heavy metals in plants, CN ratio and yield of annual ryegrass cultivated in paper mill waste amended with and without biochar application (0 and 20 Mg ha^{-1}) grown under controlled environment conditions. Heavy metals_S represents heavy metals observed in soil amended wood ash, sludge, and wood ash + sludge (Cu_S, Pb_S, Cd_S, Cr_S, Ni_S, As_S); P represents heavy metals uptake in plant (Cu_P, Pb_P, Cd_P, Cr_P, Ni_P, As_P) at harvest. Control, wood ash, sludge and wood ash + sludge with and without biochar application were used as growth media for kale production. Biplot showing the relationships between growth media amendments with and without biochar and growth parameters, yield, soil pH, plant height, N uptake, residual N and heavy metal dynamics in soil-plant system in annual ryegrass.

2.3.8.2 Kale

Crop growth parameters such as plant height, chlorophyll, number of leaves, N uptake and yield were used as indicator for agronomic performance whereas heavy metals were used as indicator for soil and plant quality. Redundancy analysis (RDA) explained 99.61 % variability of the data set where axis 1 and 2 accounted for 99.8% and 0.73% of the total variability (Figure 2.10). Bi plot expressed segregation of soil amendments into different quadrants based on observed variables (Figure 2.10). There are three distinct groups of paper mill waste and two groups of BC treatments (Figure 2.10). RDA biplot showed relationship of different observed variables affected by soil amendments (Figure 2.10). Observation plot expressed grouping of WA and WA + SL treatments due to observed variables in biplot including plant height and yield (Figure 2.10). Heavy metal concentrations were clearly explained by RDA bi plot. Soils treated with BC 20 retained heavy metals in soil and expressed lower heavy metal uptake in growing plants (Figure 2.10). Overall, RDA indicates that WA application increased kale growth and yield, whereas biochar addition at the rate of 20 Mg ha^{-1} increased heavy metal concentration in soil and decreased their uptake in kale shoots.

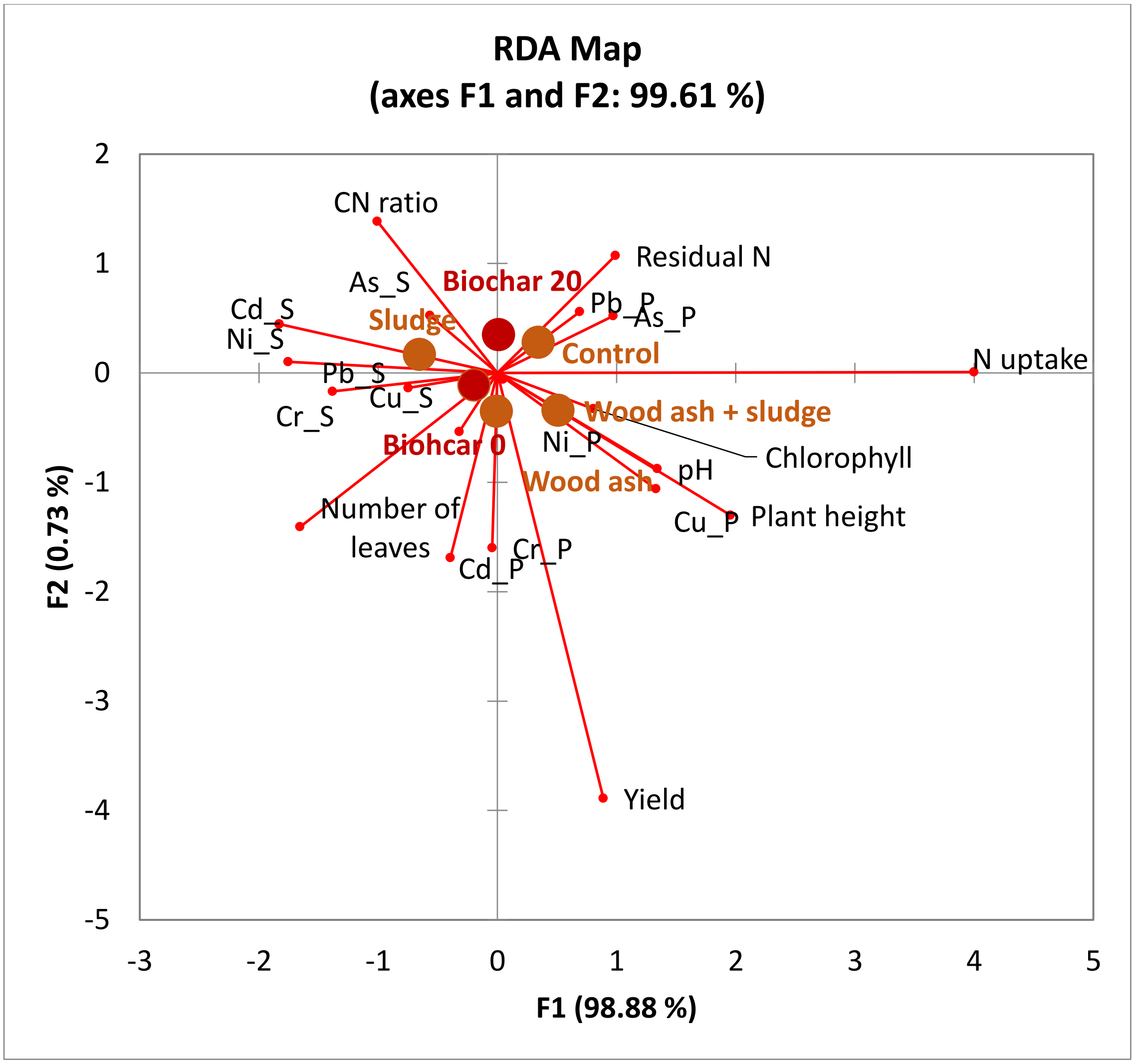

Figure 2.10: Redundancy analysis (RDA) of the growth parameters, heavy metals in soil, heavy metals in plants, CN ratio and yield of kale cultivated in paper mill waste amended with and without biochar application (0 and 20 Mg ha^{-1}) grown under controlled environment conditions. Heavy metals_S represents heavy metals observed in soil amended wood ash, sludge, and wood ash + sludge (Cu_S, Pb_S, Cd_S, Cr_S, Ni_S, As_S) and heavy metals_ P represents heavy metals uptake in kale plant (Cu_P, Pb_P, Cd_P, Cr_P, Ni_P, As_P) at harvest. Control, wood ash, sludge and wood ash + sludge with and without biochar application were used as growth media for kale production. Biplot showing the relationships between growth media amendments with and without biochar and growth parameters, yield, soil pH, plant height, N uptake, residual N and heavy metal dynamics in soil-plant system in kale.

2.3.9 Relationships among pH, yield, residual soil nitrogen, and nitrogen uptake

There were significant moderately-strong negative correlations between RSN and annual ryegrass biomass yield (r = -0.61; Figure 2.11a) and N uptake (r = -0.49; Figure 2.11b). On the other hand, pH was positively correlated with N uptake (r = 0.53; Figure 2.11c).

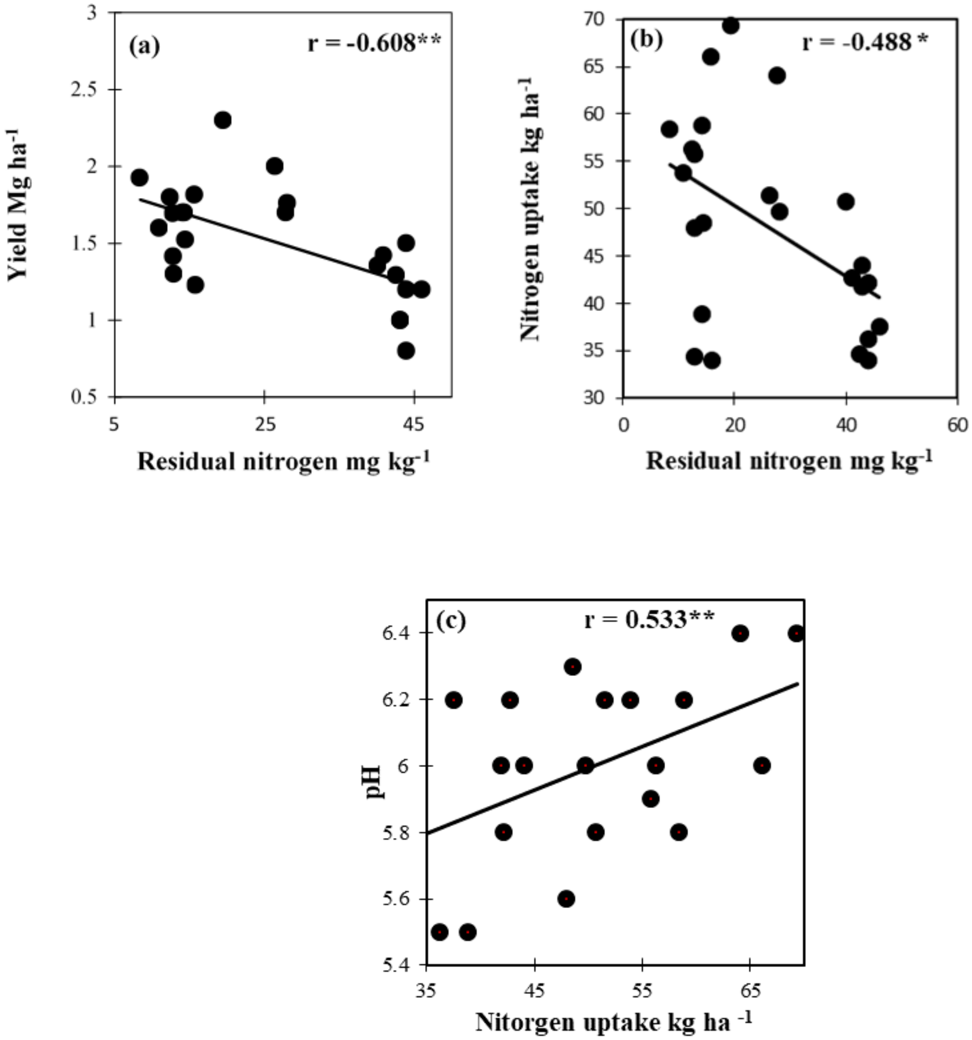

Figure 2.11: Relationships between (a) DMY and residual soil nitrogen, (b) nitrogen uptake and residual soil nitrogen, and (c) pH and nitrogen uptake. *significant at $p \leq 0.05$; **significant at $p \leq 0.01$

2.4 Discussion

Industrial wastes, as soil amendments, are getting attention of researchers, academia, environmentalists, policy makers and other industry stakeholders to investigate their effects on soil physiochemical properties, liming and soil organic carbon potential and heavy metals risk and their effects on crop growth, yield, and phytonutrients (Sharifi et al., 2013). Here, we evaluated the effects of CBPPL waste, such as WA, SL and combine application of WA and SL as potential soil amendments to enhance soil pH, crop growth, yield and heavy metal dynamics of annual ryegrass and kale under controlled environmental conditions.

2.4.1 Effects on seedling emergence, growth, yield and nitrogen dynamics of annual ryegrass and kale

Seedling emergence and development are significant steps in crop establishment and are mainly affected by environmental factors such as soil salinity, light, moisture, and temperature (El-Keblawy et al., 2017). Besides these requirements, seedlings may also be sensitive to other factors such as presence of heavy metals in the growth medium which can inhibit seed germination following seedling establishment (Bae et al., 2016). In current study, adding WA, SL or WASL did not reduce seedling emergence compared to LIME and this finding is in line with the results reported by Nabeela et al. (2015) and Ríos et al. (2012), who reported no effects of WA and SL application on seedling establishment of *Brassica napus L.* and *Lactuca sativa* L., respectively. The lack of seedling toxicity in soil amended with WA, SL, WASL, or BC can be attributed to lower concentrations of heavy metals in soil and amendments used in this study. Also, the pH and EC of amended soil in this study were within the optimum ranges as reported by Thornton (2019) (pH = 5.5 - 6.0 and EC = 2.0 - 3.5 dS m^{-1}) for plant growth under controlled environmental conditions.

Wood ash is known to be a good source of macronutrients such as potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), and micronutrients (Adekayode & Olojugba, 2010; Demeyer et al., 2001; Kukier & Sumner, 1996; Naylor & Schmidt, 1986; A. Saarsalmi et al., 2001; Sharifi et al., 2013). WA has the potential to enhance the bioavailability of plant nutrients by raising soil pH (Hakkila, 1989; Mbah et al., 2010; Naylor & Schmidt, 1986; Ohno & Erich, 1990; Sharifi et al., 2013). WA application also increased plant height compared to limestone (Kikamägi et al., 2013). In the present study, enhanced plant height of kale in WA amended treatment (Table 2.5) was observed. Enhanced plant height in annual ryegrass and kale crops could be attributed to the presence of essential nutrients e.g., Ca, Mg K, Mo, Fe, and Zn in WA (Table 2.1) that facilitate kale growth resulting in increased plant height. WA used in this study contains abundant K^+ and Ca^{2+} (Table 2.1) that might have helped in activation of various enzymes, stabilize plant metabolism and improve the plant growth performance (Hasanuzzaman et al., 2018; Lecourieux et al., 2006; Wu et al., 2018). Additionally, K^+ stabilizes protein synthesis process, involves in the osmoregulation and cytoplasmic pH homeostasis in plant cells (Sikder et al., 2020). Ca^{2+} as essential macro-element plays a vital role in cell division and enlargement, provides rigidity to the cell wall and act as secondary messenger in signal transmission (Lecourieux et al., 2006).

In present study, we observed that BC addition significantly reduced chlorophyll in annual ryegrass (Table 2.4) and these results are in line with the results reported by Fascella et al. (2017), who observed that BC addition with sphagnum peat decreased chlorophyll in *Rosa rugosa*. Decrease in the chlorophyll content in annual ryegrass shoots might be attributed to higher C/N ratio that result in N immobilization. In present study, BC addition increased C/N ratio when applied to the soil used during annual ryegrass cultivation, that may lead to reduced N uptake (Table 2.4). This observation is in line with Alburquerque et al. (2013) who reported, BC addition decreased chlorophyll and N uptake in *Triticum aestivum* that might be due to high C/N ratio of BC. As N is an essential constituent of chlorophyll and lower N uptake might account for the reduced chlorophyll in annual ryegrass due to increase in C/N ratio following BC amendment in the growth media. Deenik et al. (2010) reported that BC contains volatile fractions that can be readily degradable and may increase C/N ratio in soil.

In addition Mg^{+2} and Fe^{+3} are involved in synthesis of chlorophyll structure as well as biological nitrogen fixation (Bonfim-Silva et al., 2017). Farhangi-Abriz and Torabian (2018) reported, BC addition significantly improved K^+ uptake in the plant shoots. Higher K^+ in shoots might cause antagonistic effect and reduce Mg^{2+} uptake that eventually decrease chlorophyll (Xie et al., 2020)

In present study, we observed non-significant effect of mill amendments (WA, SL and WASL) on photosynthesis rate irrespective of biochar addition in annual ryegrass (Figure 2.2). This might be due to same nutrient level and growing conditions across the treatments. However, among non-amended BC treatments WA exhibited lower photosynthesis rate than control (Figure 2.2). In addition, BC amended control showed decreased photosynthesis rate as compared to higher rate in control without BC application (Figure 2.2), that might be due to increased C/N ratio and immobilized N which might have led to lower N uptake and decreased chlorophyll and eventually photosynthesis rate (Table 2.4). Croft et al. (2017) reported a strong linear relationship ($r = 0.78$) between leaf chlorophyll and photosynthesis rate. In present study, we observed higher C/N ratio and lower chlorophyll with BC addition (Table 2.4) that might be responsible in decreasing photosynthesis rate in BC amended control than non-amended BC Control (Figure 2.2).

Wood ash and SL contain significant amounts of macro- and micronutrients (Table 2.1) which are required for plant growth and biomass/yield (Pan & Eberhardt, 2011). WA amendment increased soil pH, nutrients availability and minimize the mobility of heavy metals in soil-plant system (Ochecova et al., 2014). Additionally, WA and SL application in soil increased organic matter, improved P uptake and other nutrients due to increased soil pH and increased biomass of *Lolium perenne* L. (Schiemenz & Eichler-lo, 2010; Ríos et al., 2012). Similarly, Kumar and Chopra (2014) observed increase in root length, stem and yield of *Phaseolus vulgaris* L. with SL amendment. In the present study, WA, SL and WASL produced 71%, 30% and 48% higher biomass of annual ryegrass than control (Table 2.4). This increased biomass in annual ryegrass could be attributed to availability of essential nutrients, particularly Ca and K improved cell division, cell wall expansion and activated various enzymes that stabilized plant metabolism and resulted in higher growth and yield (Reddy & Reddy, 2004; Weissert & Kehr, 2017). Additionally, WA and SL amendment increased soil pH, organic matter, moisture contents, microbial activities, which led to rapid mineralization of nutrients to plants and enhanced plant biomass (Couch et al., 2020; Kinnula et al., 2020). The observed higher yields in ryegrass and kale in WA amendment are in agreement with the studies conducted by Couch et al. (2020) and Kinnula et al. (2020). We also observed higher N uptake in both crops in WA amendment than control (Table 2.4 and 5) which was also confirmed by Brod et al. (2012) who reported that WA mixed with inorganic fertilizer increased nitrogen uptake in *Lolium perenne* L. In present study, higher N uptake enhanced yield of annual ryegrass (Figure 2.11 A). Higher pH of WA (i.e, 12) has the potential to create alkaline conditions in substrate with the addition of soluble bicarbonates into soil. In the result of this process, negatively charged elements become dominant e.g., (NO_3^-) and more readily soluble in soil solution as compared to positive charge elements e.g., (Al^{3+}), caused more N uptake and leads to higher biomass production in WA treatment than control (Figure 2.11 C) (Neina, 2019). Our results are in agreement with the findings of Ziadi et al. (2013), Bonfim-Silva et al. (2017), Cruz-Paredes et al. (2017) and Vestergård et al. (2018) who reported that WA, and SL application increased plant growth and biomass compared to control.

Wood ash application along with N fertilizer enhanced N uptake in *Pinus sylvestris* and reduced residual N (NH_4^+ and NO_3^-) in soil (Anna Saarsalmi et al., 2014). In the present study, higher N uptake and lower residual N (NH_4^++NO_3^-) was observed in WA treatments as compared to control (Figure 2.4Figure 2.), these results are in agreement with studies done by Couch et al. (2020), who reported that application of high carbon ash increased mineralization of C and N via microbial respiration that results in more N availability for plants and less residual N. Pugliese et al. (2014) reported, increased NO_3^- availability was observed after application of high carbon ash in a laboratory incubation experiment.

However, lower N uptake in plant tissues of both crops and higher residual nitrogen was observed in SL treatments without BC addition (Table 2.4 and 5). These results were consistent with the study done by Sippola et al. (2003), who reported lower nitrogen uptake by plants amended with paper mill sludge, however, N was adjusted as same as inorganic fertilizer source still it was not completely available to the plant. This may be due N mineralization in sludge was not synchronized with crop needs over time. In current study, SL with higher C/N ratio (38.4: 1) apparently immobilized soil mineral N, resulting in lower uptake (Table 2.4 and 5) and more residual nitrogen in soil used for annual cultivation (Figure 2.11 D) (Table 2.4). Similarly, BC addition in this study increased residual N concentration in soil used for annual ryegrass production and might be due to higher C/N ratio of BC amended treatments (Table 2.4 and 5). Increased C/N ratio in the BC amended soil may lead to N immobilization and could enhanced residual N (Luo et al., 2018).

2.4.2 Effects of soil amendments and biochar on soil pH and heavy metal concentration in soil and uptake in annual ryegrass and kale

Wood ash and SL are the potential alternate liming material that can be substituted with commercial limestone. In the present study, we observed increased soil pH with WA application compared to SL and commercial limestone material (Table 2.4 and 5). SL used in the present study was not as effective in enhancing soil pH, possibly due to low pH, large particle size and slow decomposition rate. WA is more efficient in increasing soil pH considering its high alkalinity (pH 12), and superior solubility in water compared to other liming sources (Sharifi et al., 2013). Increased soil pH might be due to presence of carbonates, oxides and hydroxides which react with water and segregate to form OH^- or HCO_3^- and Ca^{2+}, Na^+ and K^+ ions (Neina, 2019). The OH^- or HCO_3^- neutralizes H^+ in the soil solution and increase OH^- concentration that increase soil pH (Babayemi et al., 2010). Moreover, in soil particles H^+ ions present at exchangeable sites also get soluble in soil solution and Ca, Na, and K ions take that vacant spaces at exchangeable sites (Kapembwa et al., 2020). In addition, WA contains small particle size that can react with soil and change pH more rapidly than other liming materials (Hu et al., 2020). Adotey et al. (2018) observed increased soil pH with WA application as compared to commercial limestone. Whereas

SL contains nitrogen in ammonium form that might have released proton (H^+) during nitrification which enhanced soil acidity and result in low pH (He et al., 2021).

WA and SL also contain some heavy metals, hydrocarbon and other organic compounds that are toxic for plant growth and development/agriculture production and directly or indirectly could negatively affect animals and human health through contaminated feed and food (Augusto et al., 2008). WA and SL contains heavy metals such as Cr, Cd, Pb, Ni, and As that can be toxic if present in excess quantities/above than threshold level (Seshadri et al., 2015). These essential and non-essential heavy metal elements are non degradable, causing bioaccumulation in crops and can effect human health (Pateriya et al., 2021). Heavy metal concentration in soil amendments used in this study were well below the allowable limits for biosolids application (Table 2.1) (Monfared-Heidarey., 2011). In the current study, concentrations of Ni and As were not affected by WA, SL and WASL when compared with Control although Pb and Cd was observed more in SL than Control treatment and that might be due to higher application rate SL (55 Mg ha^{-1}) as compared to WA (17.25 Mg ha^{-1}). Gagnon & Ziadi (2021) reported that mineralization of the paper mill biosolid over time may gradually release heavy metals in bioavailable form. SL used in this study had high C/N ratio, may cause more mineralization that could release more bioavailable heavy metalloids over time. Whereas BC addition significantly increased Cr and As concentration in soil. These findings are in line with results reported by Koetlisi & Muchaonyerwa. (2019) in which pine wood biochar significantly adsorbed Cr in soil. This might be due to large surface area and high cation exchange capacity (CEC) of BC that could help in the adsorption of Cr and As (Medyńska-Juraszek & Ćwieląg-Piasecka, 2020). However, overall concentration of heavy metal in soil of annual ryegrass amended with WA, SL and their combination WASL for Cr, Ni As, Pb and Cd

(64, 50, 12, 70 and 1.4 mg kg^{-1}) concentration was below the allowable limit established by Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007). Whereas heavy metal concentration in annual ryegrass aboveground biomass was not significantly different when compared to the control that may be due to lower level of heavy metal concentration in the amendments (Table 2.1). In addition, Taylor et al. (2015) reported that annual ryegrass is known to be an excluder plant that prevents translocation of heavy metals from the roots to shoot parts of the plant. Roots of annual ryegrass act as barriers in the translocation of Pb possibly through precipitation into lead phosphate after transport into the cell (Rodr et al., 2019). However, in this study, metalloids concentration in shoots of annual ryegrass were below the permissible limit for forage(As 2 mg kg^{-1} and Pb , 10 mg kg^{-1}) (Rodr et al., 2019). Similarly, Cr level was below 5-100 mg kg^{-1} as reported by Oliveira (2012), Ni (10 mg kg^{-1}) and Cd (1 mg kg^{-1}) were below the allowable limit that can be toxic for annual ryegrass growth (Osmani et al., 2015).

In kale crop, more concentration of Cd was observed in WA, SL and WASL as compared to Control in soil used for kale cultivation, these results agree with studies done by Bolan et al. (2003) in which authors reported that biosolid application decrease insoluble and exchangeable cadmium but increase in organic bounded Cd concentration in soil. This might be due to a formation of Cd complex with organic matter in biosolids. Heavy metal (Cr and Pb) concentration in kale shoots by amendment application was not different when compared with control potentially due to low application rates of amendments (Table 2.1). Radulescu et al. (2013) reported poor accumulation of heavy metal in *Brassica oleracea L.* of Pb in shoots, however, higher concentration was observed in roots that might be possible since roots are more closely in contact with growth medium than the shoots. Roots of tolerant plant are more likely to absorb heavy metals as compared to shoots (Al-akeel, 2016). However, in this study, we observed that heavy metal concentrations in kale aboveground biomass were below the permissible limits set for plant production (0.2, 2, 10 ,2 , and 1.30 mg kg^{-1} of Cd, Pb, Ni, As, and Cr, respectively) (WHO, 1996). Similarly, in kale soil heavy metals concentration were lower than the permissible limit for Cr, Ni As, Pb and Cd (64, 50, 12, 70 and 1.4 mg kg^{-1}) established by the Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007).

BC amendment restricted the uptake of Pb, Cr, and Ni in shoot tissues of ryegrass and kale and this might be due to the presence of negative surface charges on BC particles that adsorbed heavy metals reducing their uptake by plants (Whitty & Tollervey, 2013; Yousaf et al., 2017). Immobilization of heavy metals might be due to increased surface area of BC particles that consequently reduced plant uptake (Liu & Zhang., 2012). Physiochemical properties of BC influenced adsorption of heavy metals e.g., pH, particle size, type of feed stock, surface charge and functional groups (Liu et al., 2017). Yuan et al. (2011) reported that pyrolysis temperature between 300-600 °C increased surface area, negative charge and formation of functional groups e.g., $-COO^-$, -COH and -OH that helps in the adsorption of heavy metals by making organic complexes. Additionally, higher CEC of BC makes it more efficient in the adsorption of heavy metal due to presence of more exchangeable sites on the surface of BC (Harvey et al., 2011).

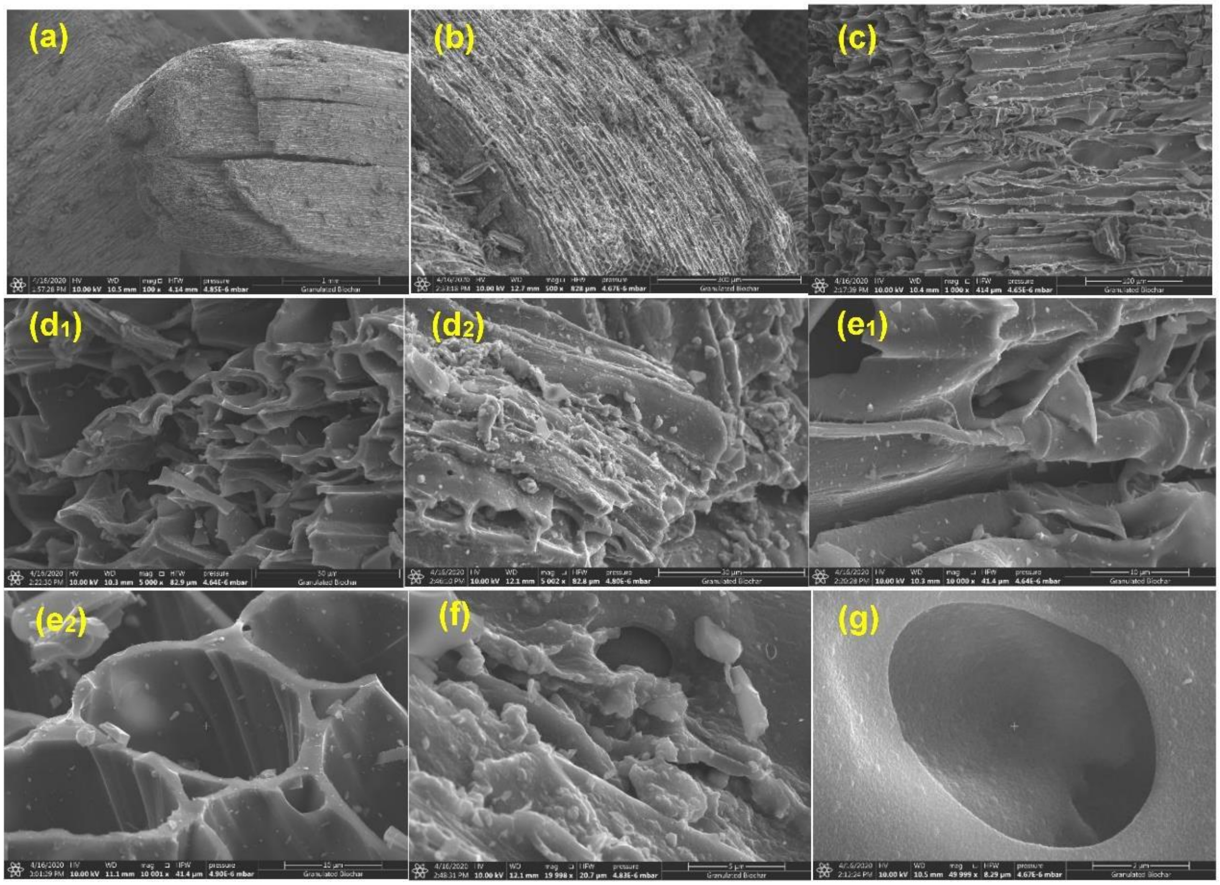

Figure 2.12 : Scanning electron microscopy image of yellow pine wood (Pinus spp.) Biochar at different magnifications and scales: (a) 100× mag—1-mm scale; (b) 500× mag—300-µm scale; (c) 1000× mag—100-µm scale; (d1) 5000× mag—30-µm scale; (d2) 5000× mag—30-µm scale; (e1) 10,000x mag—10-µm scale; (e2) 10,001× mag—10-µm scale; (f) 19,998× mag—5-µm scale; (g) 49,999× mag—2-µm scale.

BC used in this study was produced at 550 °C with high CEC, carbon, and large groves (Figure 2.12 e and g) (Table 2.3) that might have improved heavy metal sorption capacity in the soil system by making organic complexes (Uchimiya et al., 2011) and reduced plant uptake. Increase in soil pH might help in the adsorption of heavy metal to surface area of the BC and eventually reduced plant uptake. Kołodyńska et al. (2012) reported that the bioavailability of heavy metals in soil depends upon the ionization and speciation process that is strongly induced by pH variations in the biochar surface charge. Increase in pH might have contributed to the formation of functional groups present in BC that plays a major role in the adsorption of heavy metals, decreased bioavailability and uptake by plant tissues (Banik et al., 2018; Shaaban et al., 2018; Yuan et al., 2011). The results of present study are consistent with the previous study conducted by Zhang et al., (2016), who observed significant reduction in heavy metal mobility and prevent their accumulation in plant aboveground biomass with BC amendment.

2.5 Conclusion

The intent of this study was to evaluate the potential of WA, SL, and WASL as liming and nutrients source, and their effects on growth, yield, nitrogen dynamics and their combination with BC to check heavy metals mobility in soil and shoot tissues of ryegrass and kale. Results from this study demonstrate that use of WA alone and in combination with BC could potentially be used as liming and nutrient sources. As expected, WA and its combination as WASL (80 % WA + 20 % SL) increased yield of annual ryegrass and kale due to high pH and presence of essential nutrients. However, higher C/N ratio of SL might have immobilized N and eventually decreased N uptake in annual ryegrass and kale. In contrast, WA exhibited superior agronomic performance, increased soil pH, plant growth, N uptake and yield of both crops. These amendments did not exceed the heavy metals limit in soil-plant system than the permissible level and hence could be used as potential liming and nutrient sources in agricultural production systems. BC addition further decreased the heavy metal concentration uptake in plant tissues of both crops. Based on the results of this study, it can be concluded that CBPPL amendments (WA and WASL) can be used as a liming and nutrient source in acidic soils; SL application could be a useful soil amendment by lowering the C/N ratio by addition of mineral N and therefore warrants further investigation. Field research trials are required to assess and validate the liming and nutrient potential of these waste amendments on soil pH, growth, and yield and effects of BC amendment in heavy metals uptake in agronomic and horticultural cropping systems.

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Chapter 3

Evaluating paper mill biosolids and biochar effects on growth, yield, and heavy metals mobility in a low pH soil-plant system

Abstract

Paper mills burn large volume of wood for power generation to run different industrial operations. Wood ash is produced as a waste by-product of this combustion process. Whereas, SL is produced from wastewater treatment in primary and secondary clarifiers. The Corner Brook Pulp and Paper Ltd., (CBPPL) produces approximately 10,000 Mg of WA (landfilled at cox's cove) and 47,500 Mg of SL (burnt to produce steam to run different operations of paper mills). This practice is not desired due to high disposal cost and environmental concerns. Moreover, these wastes might contain heavy metals which could leach from the landfill site and may contaminate surface and ground water resources. Sustainable management of these waste could alleviate environmental and financial implications for CBPPL. One such sustainable management practice is land application of WA and SL to acidic agricultural soil, to increase soil pH, enhance crop growth and yield. Thus, a greenhouse study was conducted to evaluate the effect of WA, SL alone and in combination with BC on growth, yield, nitrogen dynamics and heavy metals mobility in soil-plant system of annual ryegrass (*Lolium perenne* L.) and kale (Brassica oleracea) under control environmental conditions. This work was conducted at the provincial Tree Nursery, Wooddale, Grand Falls-Windsor, Newfoundland. Experimental treatments were Limestone (Control), WA, SL, WASL without BC addition (0 Mg ha^{-1}) and Control, WA, SL, WASL with addition of (20 Mg ha^{-1}) BC. Limestone, WA, SL and WA + SL application rates were 8.1 Mg ha^{-1}, 20 Mg ha^{-1}, 94 Mg ha^{-1} and 13.8 + 11

Mg ha^{-1}, respectively. Results indicated that WA amendment produced 86% more biomass than control in annual ryegrass. Similarly, WA and WABC addition showed 26 and 18 % more kale yield than Control. However, in the case of SL, produced 28 % more yield than Control in annual ryegrass and 43 % lower yield than Control in kale crop SL application increased soil Pb and Cd concentrations by 19 % and 52 %, respectively, when used to cultivate annual ryegrass. WASL increased soil Ni and Cd concentrations by 21 % and 84 %, respectively, under soil used for kale cultivation. Furthermore, biochar addition decreased the shoot Pb and Cd concentration by 33 % and 50 % in kale, respectively. Results from this study suggest that WA could be used as liming and nutrient source and maybe a suitable substitute for conventional limestone application in agriculture production systems. Furthermore, BC addition with WA and SL may be helpful in adsorbing heavy metals in soil and could prevent bioaccumulation in plant tissues.

3.1 Introduction

Corner Brook pulp and paper mill limited (CBPPL), generate 10,000 Mg of WA and 47,500 Mg of SL as waste by products each year. Currently, these biosolids are disposed of at landfill sites which cause financial and environmental implications for CBPPL. Considering the high landfill cost and environmental concerns we are interested in finding sustainable solutions or alternate options to utilize paper mill biosolids in agriculture crop production (Mozaffari & Hays, 2020). One such sustainable solution is the utilization of paper mill biosolids growth media or soil amendments for crop production. WA and SL have high pH and fair amount of mineral nutrients which can be used as liming and nutrient sources in acidic and low fertile soils (Bang-Andreasen et al., 2021). Several studies have shown that WA and SL might be used as soil amendments for agriculture production. Mbah et al. (2010) reported, application of WA increased soil pH, plant height and yield of *Zea mays* L..WA and SL comprised of carbonates, bicarbonates and base cations that favours increase in soil pH (Royer-Tardif et al., 2019). An incubation study conducted by Torkashvand (2010) reported, increase in soil pH with the application of SL. In addition, WA and SL contain essential nutrients that promote plant growth and development (Kapembwa et al., 2020; Sahoo et al., 2021). However, WA and SL as industrial wastes may contain some heavy metals due to utilization of used oil during timber combustion process for power generation. During the burning process, lighter elements volatilize and condensed simultaneously to become the part of WA (Sarenbo, 2009; Wang et al., 2020; Zhong et al., 2015). Heavy metals are non-biodegradable elements that persist in environment for a longer time (Ackova, 2018). Higher concentration of heavy metals in WA and SL limits their use in agriculture production due to toxicity. Soil amendments such as a biochar (BC) could potentially absorb heavy metals in soil and reduce their uptake in plants thereby preventing heavy metals from entering into the food chain (Wang et al., 2017).

In addition, approximately 60 % of NL soils are podzolic, which are recognized to have low fertility and soil pH (Sanborn et al., 2011). Low soil pH depends upon various factors including, parent material, soil texture, mineral content and climate. For instance, soil pH decreases overtime due to weathering and leaching of the minerals caused by continuous rainfall (USDA, 2014). Different soil types posses different soil pH range due to their physical and chemical attributes. For example, soil consisting of high organic matter and clay content has strong buffering capacity and is more resistant to change in pH than sandy soils (Curtin & Trolove, 2013). Whereas sandy soil consists of low organic matter content, resulting in lower buffering capacity and water-holding capacity. Therefore, they are more prone to acidification (Minhal et al., 2020). Hence, limestone or soil amendments (WA, SL and WASL based on calcium carbonate equivalent) application are required to increase soil pH that can facilitate nutrients mobilization from soil to the plants for better growth and to prevent leaching of essential nutrients (Karaivazoglou et al., 2007; Laxminarayana et al., 2011).

Liming requirements may vary and depends on soil type, texture, initial soil pH, target pH and buffering capacity of the soil (Singh et al., 2008). For instance, protonation/ deprotonation of acid groups occurs in organic matter, therefore soil with high organic matter and buffering capacity requires more calcium carbonates to neutralize acids than a sandy soil with low organic mater and lower buffering capacity (Caballero et al., 2019). Similarly, soil pH and organic matter content significantly affect heavy metal mobility in soils. In general, heavy metal mobility is due to adsorption and desorption which depend on soil attributes such as soil pH, organic matter and cation exchange capacity (CEC) (Usman et al., 2008). Different studies reported negative correlation between soil pH and heavy metal mobility in soil. For example, a decrease in soil pH increased heavy metal desorption from soil and became available in soil solution (Sukreeyapongse et al., 2002; Zhao et al., 2010). The rational of conducting this study was to assess the efficiency of biosolids (WA, SL and WASL) as soil amendments on soil pH, growth, yield, and heavy metal mobility in the plant-soil system in a low soil pH with high organic matter and high-buffering capacity. The soil used in this study had lower pH (5.2) and higher organic matter (4.5%) than the soil with pH 5.7 and lower organic matter (2.7%) used in chapter 2 for annual ryegrass and kale cultivation under greenhouse conditions. Herein, we hypothesized that WA, SL alone and in combination with BC will raise soil pH, improve growth, yield and reduce heavy metals mobility in ryegrass and kale grown in a low pH soil with high organic matter. To test this hypothesis, a greenhouse experiment was conducted with the following objectives:

i. To investigate the effects of WA, SL alone and in combination with BC on seedling emergence, growth, and yield of annual ryegrass and kale in a low pH soil with higher organic matter.
ii. To examine the effects of WA, SL alone and in combination with BC on soil pH, N uptake and heavy metals uptake in annual ryegrass and kale.

3.2 Materials and methods

3.2.1 Site description and treatment preparation

Soil used in this study was collected from a newly cleared forest land located at Wooddale Provincial Tree Nursery, Grand Falls-Windsor, NL, Canada during 2019. Thereafter, collected soil was air dried, sieved with 4 mm mesh, and homogenized. A composite subsample of homogenized soil was sent to the Soil, Plant and Feed Laboratory, Department of Fisheries, Forestry and Agriculture, St. John's, NL for soil analysis. Soil and biochar analysis reports are presented in Table 2.1 and 2.3 of chapter 2.

3.2.2 Experimental design and treatment setup

A greenhouse experiment was conducted at Wooddale provincial tree nursery, Grand falls Windsor, NL. The experiment was setup in a randomized complete bock design with four amendments (limestone, WA, SL and WASL) and two biochar rates (0 and 20 Mg ha^{-1}) with three replications. WA, SL alone and combine applications of WA and SL and BC were amended with soil. Experimental treatments were Limestone (Control), WA, SL, WASL without BC addition (0

Mg ha^{-1}) and Control, WA, SL, WASL with addition of (20 Mg ha^{-1}) BC . Limestone, WA, SL and WA + SL application rates were 8.1 Mg ha^{-1}, 20 Mg ha^{-1}, 94 Mg ha^{-1} and 13.8 + 11 Mg ha^{-1}, respectively. WA SL and WASL rates were calculated based on the calcium carbonate equivalent (CCE) and targeting soil pH 6.3 (Equation 1).

$$\text{Application rate} = \frac{\text{Area} \times \text{Lime requirement (Mg/ha)}}{\text{CCE} \times (100 - \%\ \text{Moisture content})} \qquad \text{(Eq. 1)}$$

Different rates (100%, 50% and 25%) of the amendments (WA and SL) were analyzed with a pH test to determine the optimum rate that can attain the target pH of 6.3. Results of the pH test are presented in Table 3.1.

Table 3.1: Soil properties used in this study and pH results obtained from the application of soil amendments with different rates on weight basis

Soil		Wood ash rate (t ha^{-1})	pH	Paper sludge rate (t ha^{-1})	pH
pH	5.2	100 % (82.6)	6.9	100 % (94)	**6.2**
Organic matter	4.5	50 % (41)	6.6	70 % (47)	5.9
Arsenic	10	25 % (20.6)	**6.3**	25 % (23.5)	5.7
Cadmium	0.061	-	-	-	-
Chromium	31	-	-	-	-
Cobalt	8.2	-	-	-	-
Copper	13	-	-	-	-
Lead	8.7	-	-	-	-
Mercury	0.63	-	-	-	-
Molybdenum	0.44	-	-	-	-
Nickel	16	-	-	-	-
Selenium	0.46	-	-	-	-
Zinc	18	-	-	-	-

Note: All units are in mgkg^{-1} for heavy metals and percent (%) for organic matter.

Soil was mixed according to the calculated amendment rates and filled in pots. Each pot was watered with 60 % water to fill the pore space and allowed to stabilize for seven days. However, due to COVID- 19 pandemic, the greenhouse facility was closed for three months, and experiment halted from March 2021 to June 2021. After this period, pots were sampled to measure soil pH. In control and SL treatments, the soil pH dropped below the target pH. Additional limestone and sludge were added to get the target soil pH (6.3) and then watered to maintain 60 %WFPS. Twenty seeds of annual ryegrass and 10 seeds of kale were sown directly into the pots. After correcting for mineral N and total P and K in amendments, fertilizer rates for each treatment were modified and fertilizer added using urea, triple supper phosphate and muriate of potash. Annual ryegrass and kale were fertilized with 180 kg N, 52 kg P and 128 kg K and with 150 kg N, 87 kg P, and 149 kg K per hectare, respectively. Fertilizers were applied following soil test report and crop requirements.

3.2.3 Soil, plant sampling, measurements and analyses

The experiment was conducted following the same procedure outlined in Chapter 2. Similarly, all parameters including growth, yield, nitrogen uptake, residual nitrogen and heavy metals concentrations in shoots and soil were determined following the same protocols given in materials and method section in chapter 2.

3.2.4 Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effect of soil amendments and BC treatments on growth, yield, residual nitrogen concentration and heavy metals in shoots and soil of annual ryegrass and kale. Normality of the data set was checked by employing Shapiro-Wilkes test. Significant means of treatment were compared with the Fisher’s least significant difference (LSD) test (α = 0.05). The data were analyzed using the Statistix 10 software package (Analytical software, FL, USA) and graphs were prepared in Origin Pro, 2021 (OriginLab

Corporation, Northampton, MA, USA). To check the overall association between soil amendments, plant growth and heavy metal dynamics, redundancy analysis (RDA) was performed using XLSTAT software (Premium 2017, Version 19.5; Addinsoft, Paris, France). Pearson's correlation coefficients were used to determine the strength of the relationships between amendments physicochemical characteristics and the nutritional as well as the agronomic performance of annual ryegrass and kale following cultivation in the amended media.

3.3 Results

3.3.1 Growth parameters

3.3.1.1 Annual ryegrass

Final emergence did not vary among the amendments as well as in biochar application (Table 3.2) however, emergence % was observed between 80 and 91 %. Soil amendments had a significant effect ($P < 0.01$) on plant height and chlorophyll of annual ryegrass (Table 3.2). WA produced taller plants (49 cm) compared to SL which produced comparatively shorter (40 cm) plants. However, SL treatment was statistically at par with control (Table 3.2). Control treatment (limestone) showed higher chlorophyll although it was statistically non-significant from WA and WASL. SL exhibited lower chlorophyll (Table 3.2).

Soil amendments significantly affected photosynthesis rate of annual ryegrass (Table 3.2). Higher photosynthesis rate (10 µmolm-^{2}s^{-1}) was recorded in control (limestone) treatment which was statistically at par with WA and WASL (Table 3.2). Whereas SL showed minimum photosynthesis rate (4.5 µmolm-^{2}s^{-1}) although it was 60 % lower than the limestone treatment (Table 3.2).

3.3.1.2 Kale

Soil amendments and BC application did not affect final emergence % and were observed between (88-95%) (Table 3.3). Soil amendments had significant effects on the plant height, chlorophyll content, and photosynthesis rate (Table 3.3). WA produced taller plants (48 cm), however, it was statistically at par with control, whereas shortest plants were observed in SL (35 cm) and were 27 % dwarfed than those produced by WA (Table 3.3). Similar patterns in chlorophyll measurement was observed; higher chlorophyll was observed in WA which was statistically at par with the control and WASL. SL showed the lower chlorophyll to 12 % lower than control (limestone) and WA respectively (Table 3.3). Similarly, WASL showed higher photosynthesis rate (19 µmolm-2s^{-1}) and SL exhibited lower photosynthesis rate (13 µmolm-2s^{-1}). SL showed 31, 27 and 23 % lower photosynthesis rate than WASL, WA and control respectively (Table 3.3).

Table 3.2 Means and analysis of variance (ANOVA) showing the effects of soil amendments, biochar and their interaction on plant growth, yield, nitrogen uptake, residual nitrogen and C/N ratio in annual ryegrass under controlled environmental conditions (3 replications and n = 24).

Source of variation	Seedling Emergence (%)	Plant height (cm)	Chlorophyll (SPAD values)	Photosynthesis rate (µmolm^{-2}s^{-1})	Yield (Mg ha^{-1})	Nitrogen uptake (kg ha^{-1})	Residual N (mg kg^{-1})	C/N ratio
Amendments (AM)								
Control	88	42 b	41 a	10.0 a	2.3 c	85 c	20 a	19 b
WA	91	49 a	37 b	8.1 b	4.0 a	128 a	3.3 b	21 b
SL	91	40 b	26 c	4.5 c	3.6 b	36 d	2.2 c	27 a
WASL	80	41 b	37 b	9.0 ab	4.0 a	97 b	2.1 c	20 b
BC (Mg ha^{-1})								
0	88	43	35	7.9	3.6	92 a	5.7 b	20 b
20	86	42	34	7.8	3.3	81 b	8.4 a	22 a
				p - value				
AM	0.08	0.04	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01
BC	0.51	0.39	0.49	0.86	0.13	<0.01	<0.01	0.02
AM x BC	0.62	0.18	0.40	0.11	0.57	0.28	<0.01	0.39

Table 3.3: Means and analysis of variance (ANOVA) showing the effects of soil amendments, biochar and their interaction on, plant growth, yield, nitrogen uptake, residual nitrogen and C/N ratio in kale under controlled environmental conditions (3 replications and n = 24).

Source of variation	Seedling Emergence (%)	Plant height (cm)	Number of leaves (plant-1)	Chlorophyll (SPAD values)	Photosynthesis rate (µmolm-2s-1	Yield (g plant^{-1})	Nitrogen uptake (mg pot^{-1})	Residual N (mg kg^{-1})	C/N ratio
Amendments (AM)									
Control	92	43 b	13 a	42 a	17 a	149 b	850 a	3.0 b	25 b
WA	91	48 a	14 b	42 a	18 a	193 a	813 a	1.8 c	27 b
SL	90	35 c	10 a	37 b	13 b	84 d	320 c	3.7 a	30 a
WASL	92	43 b	13 a	41 a	19 a	136 c	491 b	2.0 c	26 b
BC (Mg ha^{-1})									
0	88	41	13	40	16	143 a	629	2.8	26 b
20	94	44	12	41	18	137 b	608	2.4	29 a
p - value									
AM	0.95	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
BC	0.06	0.06	0.34	0.30	0.15	0.03	0.65	0.14	< 0.01
AM x BC	0.18	0.76	0.01	0.81	0.32	<0.01	0.32	0.01	< 0.01

Soil amendments and BC interaction had significant ($P < 0.001$) effects on the number of leaves of kale crop (Table 3.3). Maximum (14 leaves plant^{-1}) number of leaves were observed in WA amended with BC and minimum number of leaves was noted in SL amended with BC (10 leaves plant^{-1}) (Figure 3.1). Among non-amended BC treatments SL showed 20 % lower number of leaves compared to control, whereas in BC amended treatments SL exhibited 23 % lower number of leaves than control (Figure 3.1).

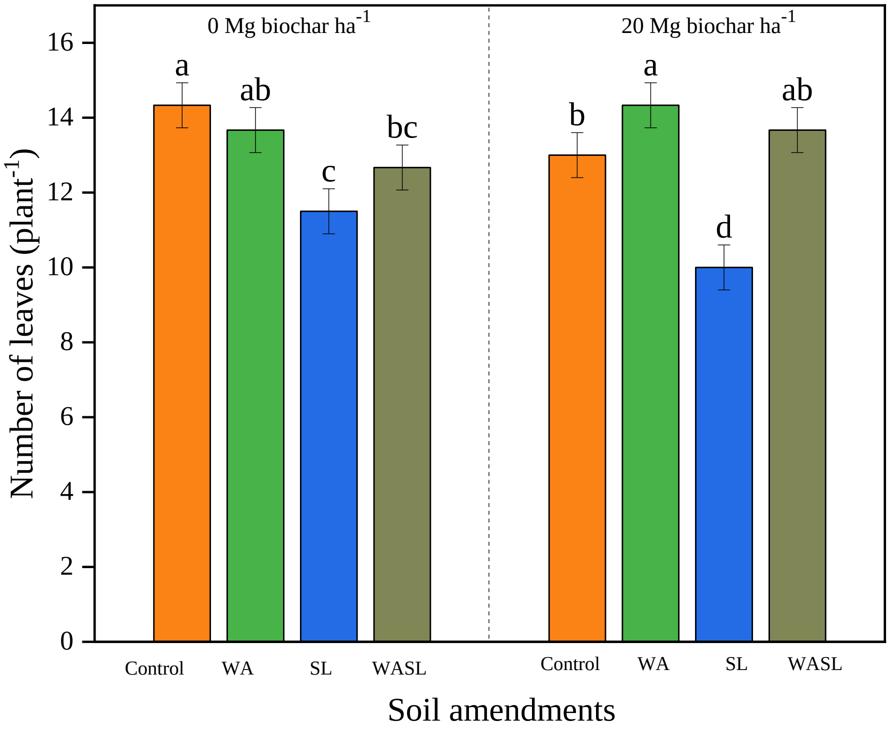

Figure 3.1: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application rates on number of leaves (plant^{-1}) under controlled environmental conditions. Values in bar chart (number of leaves/plant) represent means ± standard errors. Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test, replications = 3 and n = 24.

3.3.2 Biomass yield

3.3.2.1 Annual ryegrass

Soil amendments had significant ($P < 0.001$) effects on yield of ryegrass (Table 3.2). WA produced significantly higher yield (4.0 Mg ha^{-1}) compared to control which produced lower yield (2.3 Mg ha^{-1}) (Table 3.2). Overall, WA, SL and WASL produced 86%, 72% and 86% higher biomass than control (Table 3.2).

3.3.2.2 Kale

Significant ($P < 0.001$) soil amendment × BC interaction was observed in kale yield (Table 3.3). Maximum yield (200 g plant^{-1}) was produced in WA without BC compared to lowest (73 g plant^{-1}) in SL amended with BC (Figure 3.2). WA with and without BC amendment produced higher kale yield and SL with and without BC produced lower kale yield. WA amended BC produced 18 % more yield compared to control, whereas SL amended BC treatment exhibited 51 % lower yield than control (Figure 3.2).

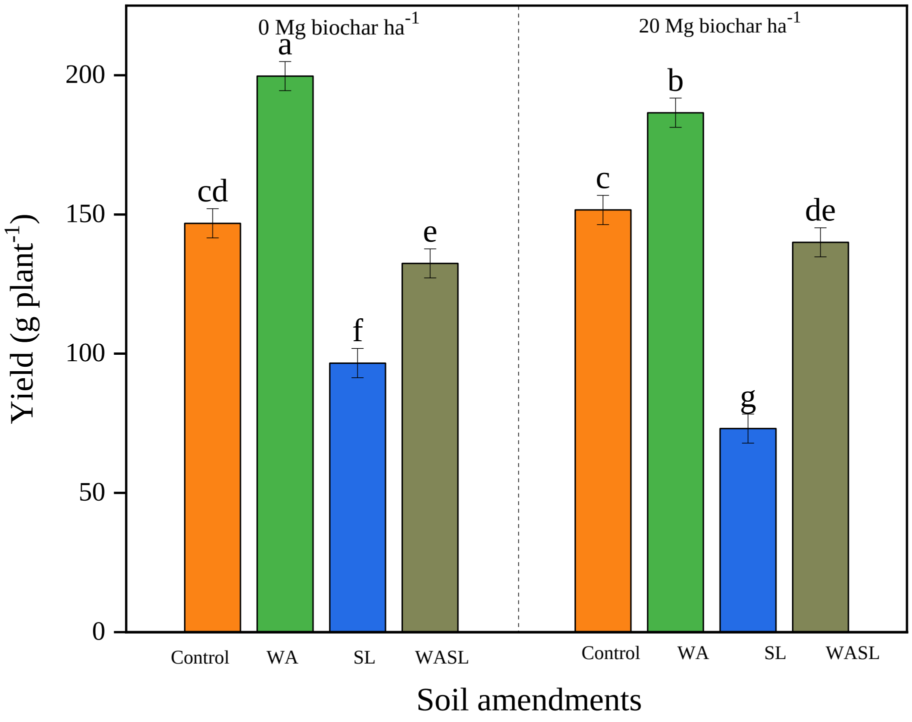

Figure 3.2: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on kale yield under controlled environmental conditions. Values in bar chart (g plant^{-1}) represent means ± standard errors (3 replications and n = 24). Bars represent the standard error for comparison. Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

3.3.3 Nitrogen uptake

3.3.3.1 Annual ryegrass

Soil amendments and BC had significant effects ($P < 0.001$) on nitrogen uptake of ryegrass (Table 3.2). We observed higher N uptake in WA (128 kg ha^{-1}), 33 % greater than the control (Table 3.2). Minimum N uptake was recorded in SL (36 kg ha^{-1}) that was 57 % lower than control (Table 3.2).

BC amended ryegrass exhibited 12 % lower nitrogen uptake than non-amended BC treatment (Table 3.2).

3.3.3.2 Kale

Soil amendments had significant ($P < 0.001$) effects on nitrogen uptake in kale crop (Table 3.3). Higher nitrogen uptake was observed in WA (813 mg pot^{-1}) although it was statistically nonsignificant with control, and lowest was noted in SL (320 mg pot^{-1}) (Table 3.3). SL and WASL exhibited 62 % and 42 % less nitrogen uptake than control (Table 3.3).

3.3.4 Residual nitrogen

3.3.4.1 Annual ryegrass

Interactive effects of soil amendments × BC had significantly ($P < 0.001$) affected residual N concentration in annual ryegrass soil (Table 3.2). Control (limestone) amended with BC exhibited significantly higher (26 mg kg^{-1}) residual N concentration whereas lowest (2.1 mg kg^{-1}) was observed in BC amended WASL (Figure 3.3). Control amended BC treatment showed higher residual N concentration compared to SL amended BC treatment. Residual N concentration in WA, SL and WASL with and without BC were statistically not different from each other (Figure 3.3). Among non-amended BC treatments WA, SL and WASL exhibited 72, 83, and 84 % lower residual nitrogen concentration compared to control, whereas in WA, SL and WASL along with BC application showed 82, 91 and 92% lower residual nitrogen than control (Figure 3.3).

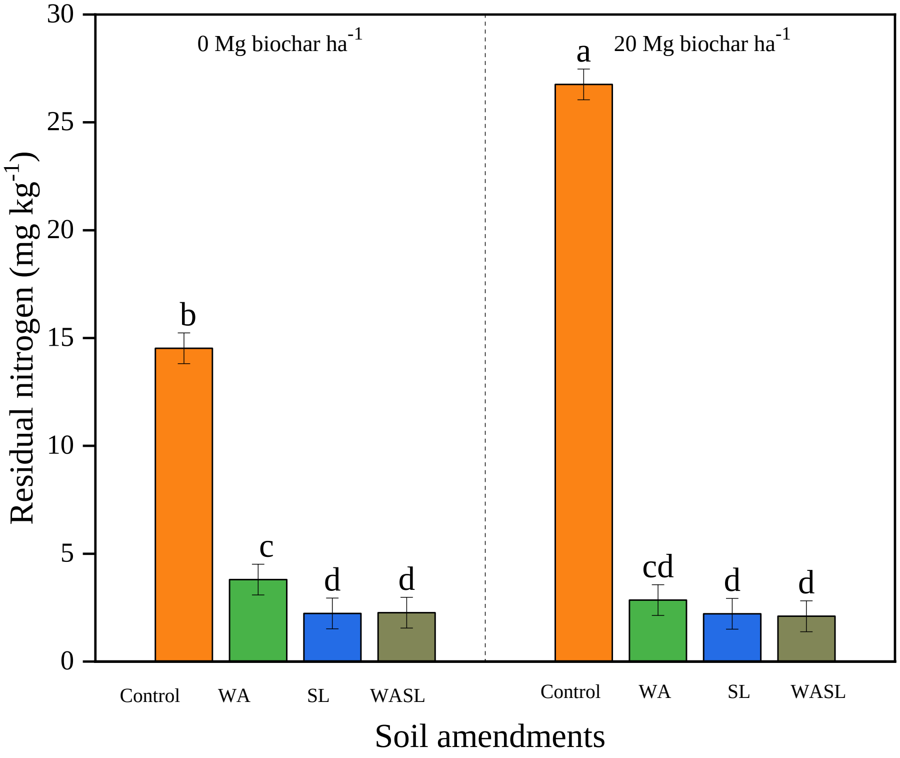

Figure 3.3: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application on annual ryegrass residual N in soil under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

3.3.4.2 Kale

Soil amendments × BC interaction had significant effects ($P < 0.001$) on residual N concentration in kale soil (Table 3.3). Non-amended BC control showed higher (3.8 mg kg^{-1}) residual N, though statistical similar with SL with and without BC amended treatments and lower (2 mg kg^{-1}) was observed in non-amended WA (Figure 3.4). Among non-BC amended treatments WA and WASL showed 57 % and 47 % lower residual N compared to control (Figure 3.4).

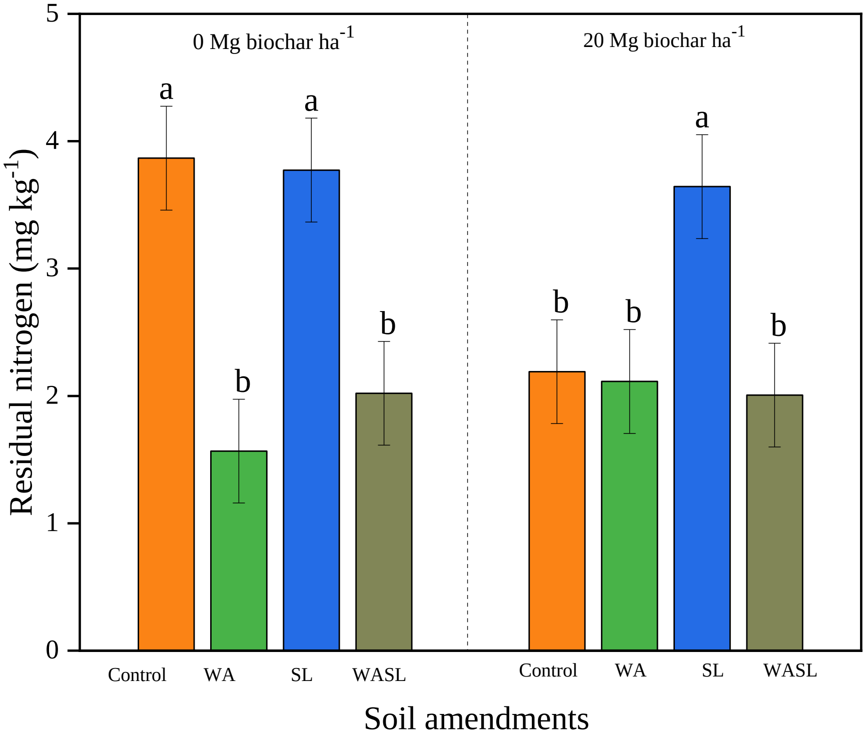

Figure 3.4: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application rates on kale residual N in soil under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

3.3.5 C/N ratio

3.3.5.1 Annual ryegrass

Soil amendments and BC significantly ($P < 0.01$, $P = 0.02$) affected C/N ratio of annual ryegrass soil (Table 3.2). Higher C/N ratio was observed in SL followed by lowest in control although it was statistically at par with WA and WASL (Table 3.2). BC amended treatments increased C/N ratio by 9 % compared to non-amendment BC treatments (Table 3.2).

3.3.5.2 Kale

Interactive effect of soil amendments × BC had significant (*P* < 0.01) effect on C/N ratio of kale soil (Table 3.3). Higher C:N ratio was observed in SL amended BC compared to lower was recorded in WASL without BC and control amended BC treatments. Treatments without BC application were statistically not different from each other including control. However, among treatments with BC application, BC amended SL exhibited 32 % higher C/N ratio compared to control amended with BC (Figure 3.5).

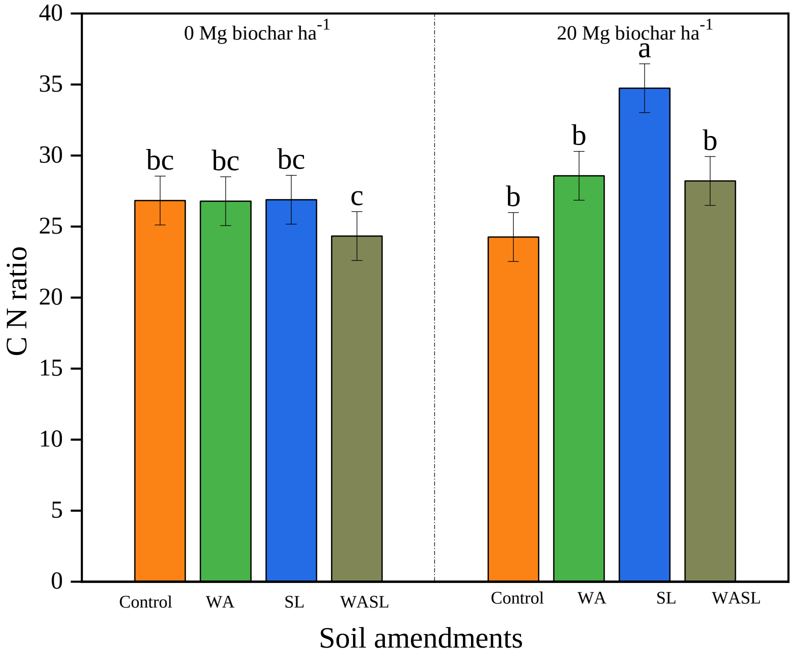

Figure 3.5: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application rates on kale residual nitrogen in soil under controlled environmental conditions. Values in bar chart (C:N ratio) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

3.3.6 Soil pH

3.3.6.1 Annual ryegrass

Soil amendments had significant effects on soil pH (Table 3.4). Higher soil pH 6.5 was observed in control, which was statistically not different from WA and WASL, and lower (6.2) was noted in SL. SL showed 5 % lower soil pH compared to control treatment (Table 3.4).

3.3.6.2 Kale

Soil amendments had significantly affected soil pH (Table 3.6). Control exhibited higher (6.6) soil pH compared to lowest (6.1) was recorded in SL (Table 3.6). However, WA and WASL effectively raised soil pH from 5.2 to 6.3 (target pH) in both crops (Table 3.6). SL showed 7 % lower soil pH compared to control (Table 3.6).

3.3.7 Heavy metals in soil and plant tissues

3.3.7.1 Annual ryegrass

Soil amendments, BC, and their interaction had non-significant on Cr concentration in soil as well as in shoot tissues (Table 3.4 and 5).

Soil amendments and soil amendments × BC interaction had no significant effect on Ni concentration in annual ryegrass soil (Table 3.4). BC amended soil significantly increased Ni concentration compared to non-amended BC soil (Table 3.4). Whereas, soil amendments, BC and their interaction had nonsignificant effect on Ni concentration in annual ryegrass shoot tissues (Table 3.5).

Table 3.4: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar on heavy metal concentration in soil of annual ryegrass crop under controlled environmental conditions (mg kg^{-1}) (3 replications and n = 24).

Source of variation	Soil pH	Cr	Ni	Cu	As	Pb	Cd
Soil amendment (AM)							
Control	6.5 a	27	14	8.6 c	7.6	7.9 b	0.08 b
WA	6.3 ab	29	12	10 b	7.6	8.3 b	0.08 b
SL	6.2 b	30	13	14 a	7.2	9.8 a	0.17 a
WASL	6.3 ab	29	13	10 b	7.5	7.9 b	0.04 c
Biochar (BC) Mg ha^{-1}							
0	6.2	28	12 b	10 b	7.3 b	8.4	0.08 b
20	6.3	29	14 a	11 a	8.0 a	8.6	0.10 a
				p - value			
AM	0.01	0.27	0.19	< 0.01	0.32	< 0.01	< 0.01
BC	0.08	0.26	0.03	0.02	0.02	0.11	0.02
AM x BC	0.53	0.98	0.51	0.93	0.86	0.34	0.26

Table 3.5: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar on heavy metal concentration in shoots of annual ryegrass crop under controlled environmental conditions (mg kg^{-1}) (3 replications and n = 24).

Source of variation	Cr	Ni	Cu	As	Pb	Cd
Soil amendment (AM)						
Control	1.4	1.7	1.1 a	0.4 b	0.5	0.07
WA	1.5	0.9	0.9 b	0.3 b	0.4	0.07
SL	1.6	1.2	0.6 c	0.4 b	0.4	0.04
WASL	2.5	2.2	0.9 b	0.9 a	0.4	0.06
Biochar (BC) Mg ha^{-1}						
0	2.1	1.8	0.9 a	0.6	0.5	0.07
20	1.4	1.2	0.8 b	0.4	0.3	0.05
				p - value		
AM	0.24	0.19	< 0.01	0.03	0.74	0.38
BC	0.09	0.13	0.02	0.06	0.06	0.12
AM x BC	0.93	0.98	0.83	0.97	0.86	0.33

Soil amendments and BC significantly affected ($P < 0.001$& 0.02) Cu concentration in annual ryegrass soil (Table 3.4). Among soil amendments SL exhibited higher (14 mg kg^{-1}) Cu concentration and lowest (8.6 mg kg-1) was observed in control. SL, WA and WASL treatments showed 28 %, 14 and 14 % higher Cu concentration compared to control (Table 3.4). Whereas BC addition increased by 9 % Cu concentration compared to non-amended BC soil (Table 3.4). Similarly, soil amendments and BC had significant ($P < 0.01$, 0.02) effects on Cu concentration in shoot tissues (Table 3.5). Among soil amendments limestone (control) exhibited higher (1.1 mg kg^{-1}) Cu concentration in annual ryegrass tissues compared to lower in SL (0.6 mg kg^{-1}), whereas BC addition reduced 11 % Cu uptake in shoot tissues compared to non-amended BC soil (Table 3.5). Soil amendments had nonsignificant effects on As concentration in soil, however, BC addition increased (9 %) As concentration in soil compared to non-amended BC soil (Table 3.4). Whereas in annual ryegrass shoots tissues, soil amendments significantly ($P = 0.03$) affected As concentration (Table 3.5). WASL showed 55 % higher concentration of As compared to WA (Table 3.5).

Soil amendments and BC application rates had significant effects on Pb concentration in annual ryegrass soil (Table 3.4). Among soil amendments maximum (9.8 mg kg^{-1}) Pb concentration was observed in SL compared to minimum (7.9 mg kg^{-1}) in control which was statistically at par with WA and WASL (Table 3.4). In annual ryegrass shoot tissues, soil amendments, BC and their interaction had no-significant effect on As uptake (Table 3.5). Soil amendments and BC significantly ($P < 0.001$, 0.02) affected Cd concentration in soil used for annual ryegrass cultivation (Table 3.4). SL exhibited higher (0.17 mg kg^{-1}) concentration followed by lower in WASL (0.04 mg kg^{-1}). Soil amended with BC increased Cd concentration in annual ryegrass soil by 20 % (Table 3.4). Whereas soil amendments, BC and their interaction had nonsignificant effects on Cd uptake in annual ryegrass shoots (Table 3.5).

3.3.7.2 Kale

Soil amendments and BC had significant ($P < 0.001$) effects on Cr concentration in soil (Table 3.6). SL showed higher (33 mg kg^{-1}) Cr concentration, which was statistically at par with WA and WASL, compared to lower (27 mg kg^{-1}) in control (Table 3.6). BC amended soil exhibited 9 % higher Cr concentration than non-amended BC soil (Table 3.6). In shoots tissues, soil amendments and BC significantly ($P < 0.001$) affected Cr concentration (Table 3.7). WA showed higher (1.1 mg kg^{-1}) Cr concentration which was statistically at par with WASL compared to lower (0.4 mg kg^{-1}) in control (Table 3.7). BC amended soil reduced Cr uptake by 45 % than non-amended BC soil (Table 3.7).

Soil amendments and BC had significant ($P < 0.001$) effect on Ni concentration in soil (Table 3.6). WA showed highest (16 mg kg^{-1}) Ni concentration, which was statistically at par with SL compared to lowest (4 mg kg^{-1}) in control. Contrarily, soil amendments, BC application rates and their interaction had no significant effects on Ni concentration in shoot tissues of kale (Table 3.7).

Soil amendments had significantly ($P < 0.001$) affected Cu concentration in kale soil (Table 3.6). highest Cu concentration was observed in SL treatment as compared to lowest in control. WA, SL and WASL showed 28, 41 and 33 % higher Cu concentration as compared to control (Table 3.6). Significant ($P < 0.001$) amendments × BC interaction was observed for Cu concentration in shoot tissues (Table 3.7). Maximum Cu concentration was observed in non-amended BC SL treatment, whereas lowest Cu concentration was noted in BC amended control. Among soil amendments without BC addition, SL and WASL showed higher Cu concentration compared to WA. Whereas among BC amended treatments, SL and WASL showed higher Cu concentration compared to WA and control (Figure 3.6).

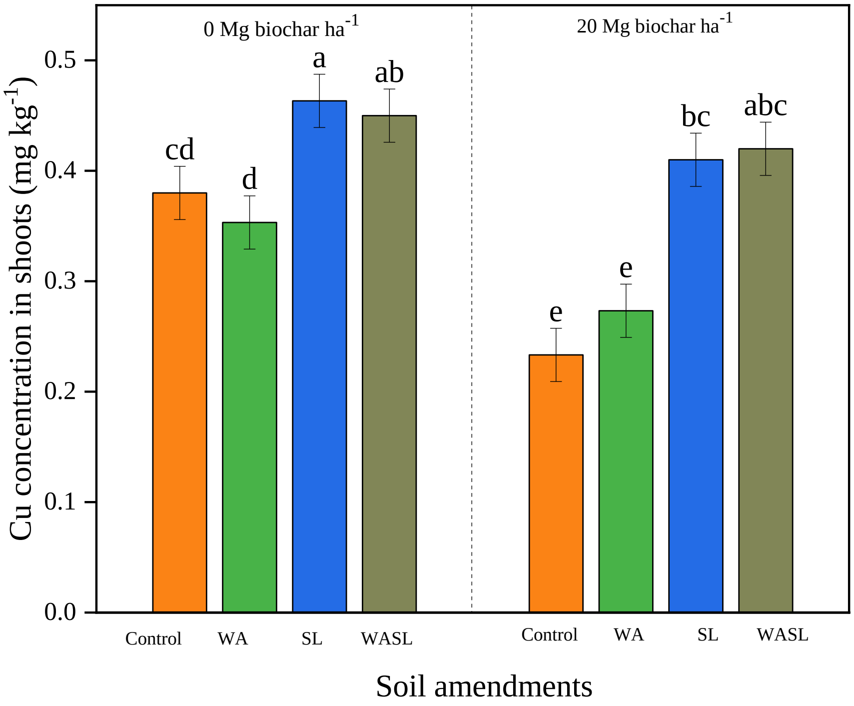

Figure 3.6: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application rates on copper concentration in kale shoots under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

Soil amendments, BC and their interaction had no significant ($P > 0.05$) effects on As accumulation in soil (Table 3.6). Inversely, soil amendment and BC significantly ($P < 0.01$) affected As concentration in kale shoot tissues (Table 3.7). Higher (0.5 mg kg^{-1}) As concentration was observed in WASL as compared to lower in control (0.3 mg kg^{-1}). WASL showed 40 % more As concentration in shoots tissues compared to control (Table 3.7). Whereas BC amended soil reduced 40 % As uptake in kale shoots (Table 3.7).

Soil amendments and BC had significant ($P < 0.01$) effects on Pb concentration in soil (Table 3.7). Higher Pb concentration was observed in WASL (9.9 mg kg^{-1}) compared to minimum in WA (7.8 mg kg^{-1}) which was statistically at par with control (Table 3.6). In addition, SL and WASL showed 21 and 22 % higher concentration of Pb in kale soil (Table 3.6). Significant ($P = 0.01$) interactive effect of soil amendments × BC was noted in Pb concentration in kale tissues (Table 3.6). Non-amended WASL exhibited higher (0.43 mg kg^{-1}) Pb uptake compared to lower (0.2 mg kg-1) was observed in BC amended SL treatment (Figure 3.7). Among non-amended BC treatments WASL showed higher Pb concentration compared to WA and control by 30 and 25 % respectively. Whereas among BC amended treatments WASL showed 35 % more Pb concentration than SL in shoot tissues (Figure 3.7).

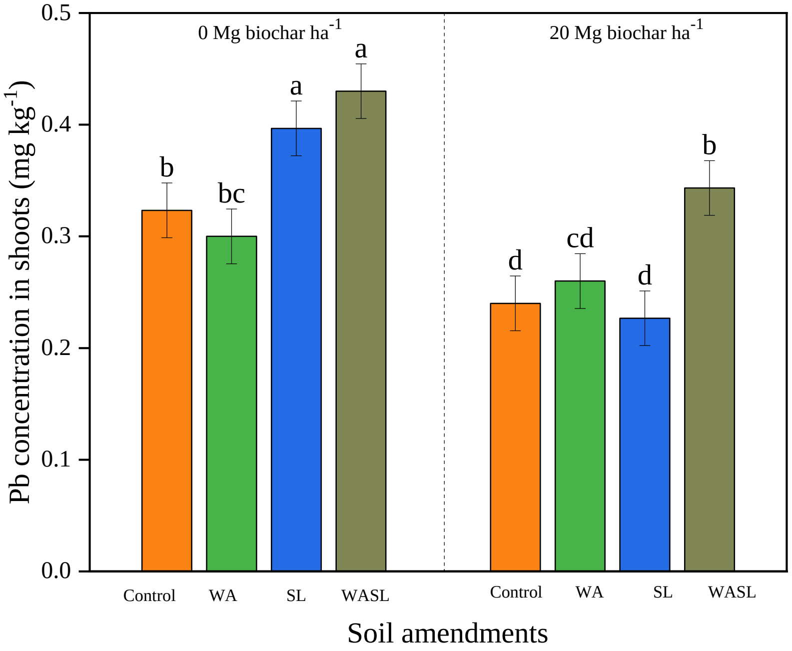

Figure 3.7: Interactive effects of limestone (Control), wood ash (WA), sludge (SL), wood ash + sludge (WASL) and biochar (BC) application rates on lead concentration in kale shoots under controlled environmental conditions. Values in bar chart (mg kg^{-1}) represent means ± standard errors (3 replications and n = 24). Values showing the same letters are not significantly different ($p < 0.05$), Fisher's LSD test.

Soil amendments and BC had significant ($P < 0.01$, 0.04) on Cd concentration in soil used for kale cultivation (Table 3.6). Among soil amendments highest (0.25 mg kg^{-1}) Cd concentration was observed in SL compared to lowest (0.04 mg kg^{-1}) in control which was statistically at par with WA and WASL. Soil amended BC treatment increased 4 % Cd concentration than non-amended BC soil (Table 3.6). Similarly, soil amendments and BC had significant ($P = 0.04$ & 0.02) effects on Cd concentration in kale shoots (Table 3.7). WASL showed highest (0.08 mg kg^{-1}) uptake compared to lowest (0.02 mg kg^{-1}) in SL. Whereas BC amended soil reduced Cd uptake by 50 % than non-amended BC soil (Table 3.7).

Table 3.6: Means and analysis of variance (ANOVA) showing the effects of soil amendments and biochar on heavy metal concentration in shoots of kale crop under controlled environmental conditions (mg kg^{-1}) (3 replications and n = 24).

Source of variation	Soil pH	Cr	Ni	Cu	As	Pb	Cd
Soil amendment (Am)							
Control	6.6 a	27 b	10 c	10 c	9.1	7.7 b	0.04 b
WA	6.2 b	30 b	16 a	14 b	9.9	7.6 b	0.06 b
SL	6.1 b	33 a	15 a	17 a	8.9	9.8 a	0.25 a
WASL	6.4 ab	33 a	12 b	15 b	7.9	9.9 a	0.05 b
Biochar (BC) Mg ha-1							
0	6.3	29 b	13	29	8.7	8.6 b	0.09 b
20	6.3	32 a	14	32	9.2	9.0 a	0.11 a
				p - value			
Am	< 0.01	< 0.01	< 0.01	< 0.01	0.054	< 0.01	< 0.01
BC	0.71	< 0.01	0.09	0.09	0.25	< 0.01	0.04
Am x BC	0.82	0.73	0.81	0.64	0.97	0.68	0.50

Table 3.7: Means and Analysis of variance (ANOVA) showing the effects of soil amendments and biochar on heavy metal concentration in shoots of kale crop under controlled environmental conditions (mg kg^{-1}) (3 replications and n = 24).

Source of variation	Cr	Ni	Cu	As	Pb	Cd
Soil amendment (AM)						
Control	0.4 c	0.4	0.3	0.3 c	0.3	0.05 ab
WA	1.1 a	0.7	0.3	0.4 ab	0.2	0.04 b
SL	0.7 b	0.6	0.4	0.4 b	0.3	0.02 b
WASL	1.1 a	0.8	0.4	0.5 a	0.4	0.08 a
Biochar (BC) Mg ha-1						
0	1.1 a	0.8	0.4	0.5 a	0.3	0.06 a
20	0.6 b	0.6	0.3	0.3 b	0.2	0.03 b
				p - value		
AM	< 0.01	0.09	< 0.01	< 0.01	< 0.01	0.04
BC	< 0.01	0.07	< 0.01	< 0.01	< 0.01	0.02
AM x BC	0.07	0.74	0.02	0.10	0.01	0.50

3.3.8 Relationship between soil amendments, agronomic performance and crop quality

3.3.8.1 Annual ryegrass

Redundancy analysis (RDA) explained 96.57 % variability of the data set where axis 1and 2 accounted for 90.13% and 6.44 % of the total variation observed in the data (Figure 3.8). RDA bi plot expressed segregation of soil amendments into different quadrants based on observed variables (Figure 3.8). RDA biplot exhibited relationship of different observed variables affected by soil amendments (Figure 3.8). Bi plot showed grouping of WA, WASL and 0 biochar treatments due to observed variables biplot including plant height, chlorophyll and N uptake. Heavy metal dynamics were clearly explained by biplot of RDA. Soils treated with BC 20 retained heavy metals and expressed lower heavy metal uptakes in growing plants (Figure 3.8). Overall, RDA indicates that wood ash application increased annual ryegrass growth and produced higher yield. Whereas biochar addition with soil amendments reduced heavy metal uptake in annual ryegrass shoots (Figure 3.8).

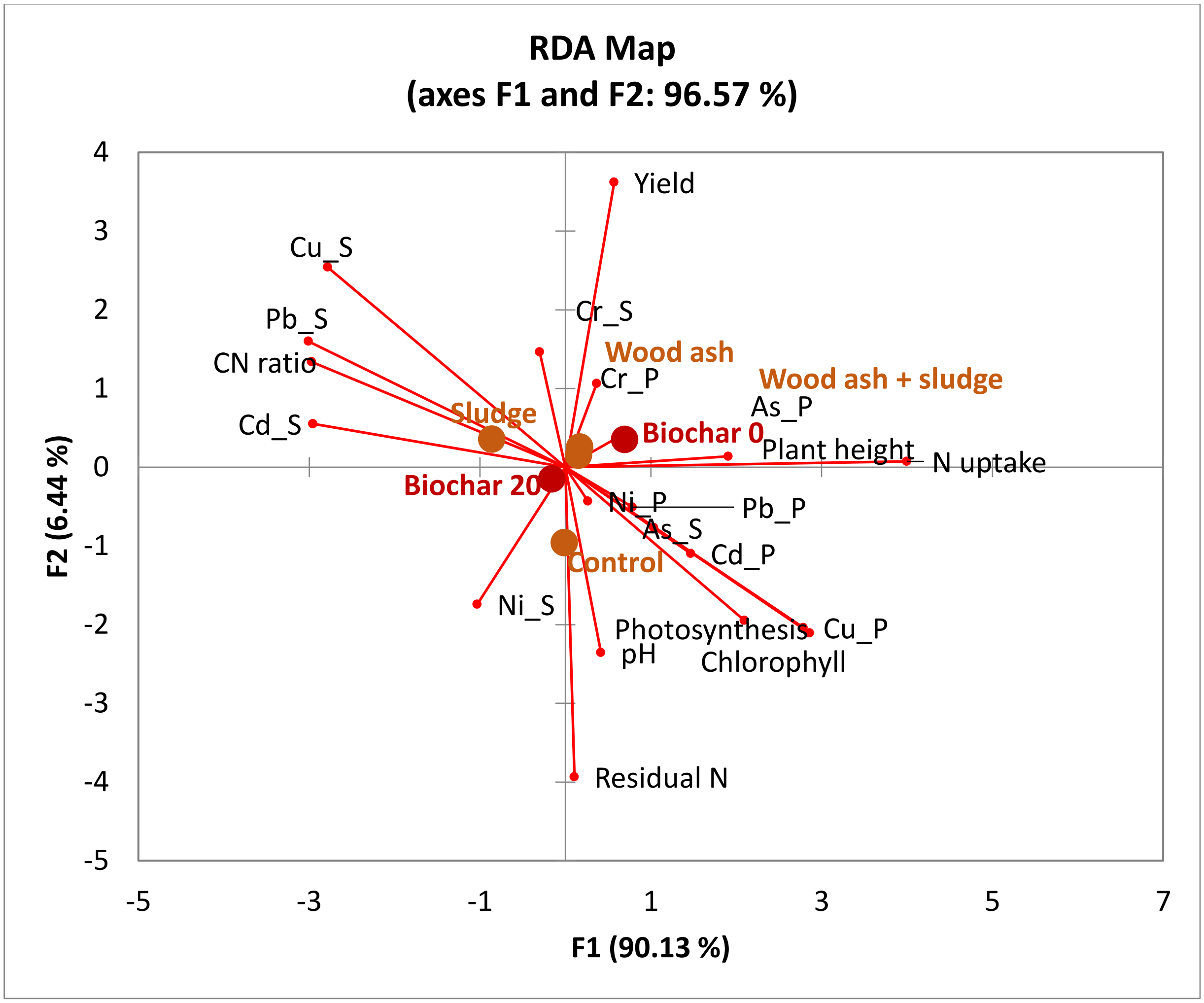

Figure 3.8: Redundancy analysis (RDA) of the growth parameters, heavy metals in soil and plants, CN ratio and yield of annual ryegrass cultivated in paper mill amended biosolids with and without biochar application (0 and 20 Mg ha^{-1}) grown under controlled environment conditions. Heavy metals_S represents heavy metals observed in soil amended biosolids (Cu_S, Pb_S, Cd_S, Cr_S, Ni_S, As_S) and heavy metals_ P represents heavy metals uptake in annual ryegrass plant (Cu_P, Pb_P, Cd_P, Cr_P, Ni_P, As_P) at harvest. Control, wood ash, sludge and wood ash + sludge with and without biochar application were used as growth media for annual ryegrass production. Biplot showing the relationships between biosolid amendments and growth parameters, yield, soil pH, plant height, N uptake, residual N and heavy metal dynamics in soil-plant system in annual ryegrass.

3.3.8.2 Kale

Redundancy analysis (RDA) explained 99.91% variability of the data set where axis 1 explained 98.74% and PC2 explained 1.17 % variability (Figure 3.9). RDA bi plot expressed separation of soil amendments into different quadrants based on observed variables (Figure 3.9). There are three distinct groups of paper mill waste and two groups of BC treatments (Figure 3.9). Observation plot expressed grouping of WA and WA + SL due to observed variables in biplot including plant height, chlorophyll and yield (Figure 3.9). Heavy metal dynamics were clearly explained by biplot of RDA. Soils treated with BC 20 retained heavy metals and expressed lower heavy metal uptakes in growing plants (Figure 3.9). Overall, this RDA indicate that WA application enhanced kale growth, nitrogen uptake and yield. In addition, biochar application along with soil amendments reduced heavy metal uptake in kale shoots and retained them in soil. (Figure 3.9).

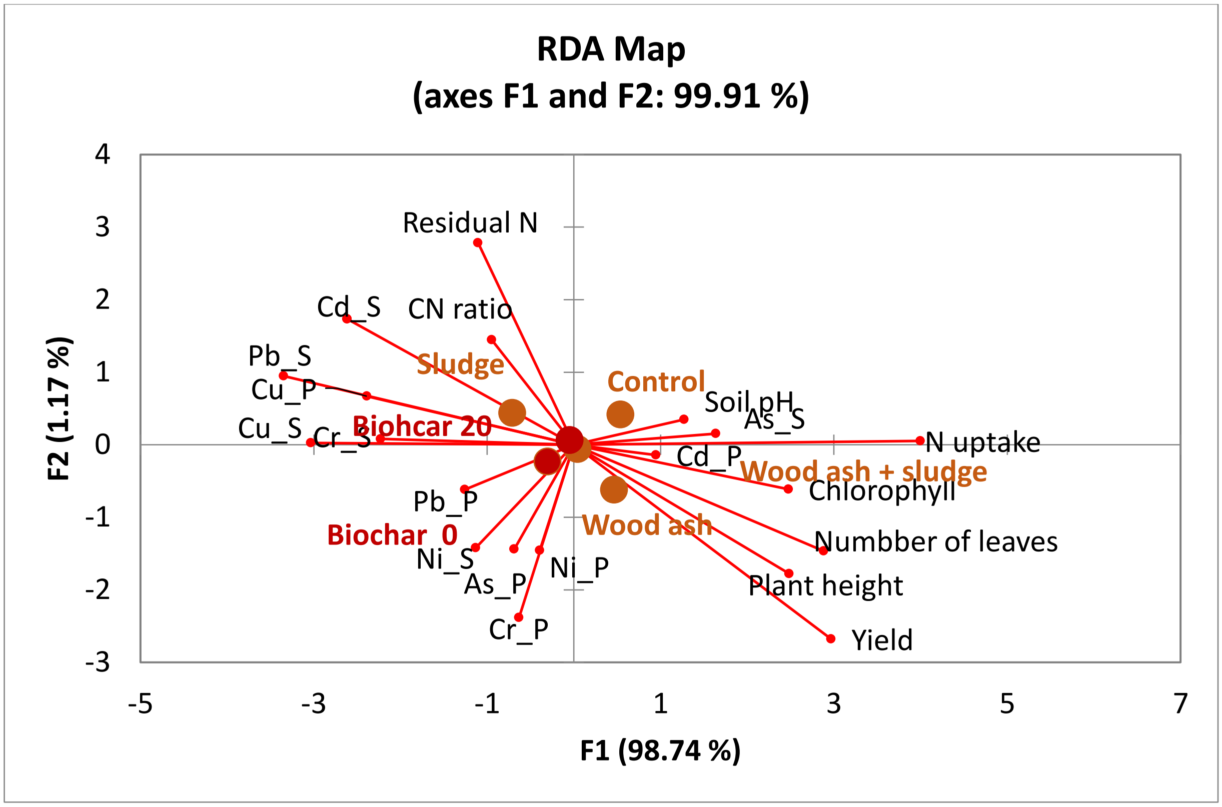

Figure 3.9: Redundancy analysis (RDA) of the growth parameters, heavy metals in soil, heavy metals in plants, CN ratio and yield of kale cultivated in paper mill amended biosolids with and without biochar application rates (0 and 20 Mg ha^{-1}) grown under controlled environment conditions. Heavy metals with_S represents heavy metals observed in soil amended biosolids (Cu_S, Pb_S, Cd_S, Cr_S, Ni_S, As_S) and heavy metals with P represents heavy metals uptake in kale plant (Cu_P, Pb_P, Cd_P, Cr_P, Ni_P, As_P) at harvest. Control, wood ash, sludge and wood ash + sludge with and without biochar application were used as growth media for kale production. Biplot showing the relationships between biosolid amendments and growth parameters, yield, soil pH, plant height, N uptake, residual N and heavy metal dynamics in soil-plant system in kale.

3.3.9.2 Relationships among chlorophyll, yield, C/N ratio residual soil nitrogen, and nitrogen uptake

3.3.9.1 Annual ryegrass

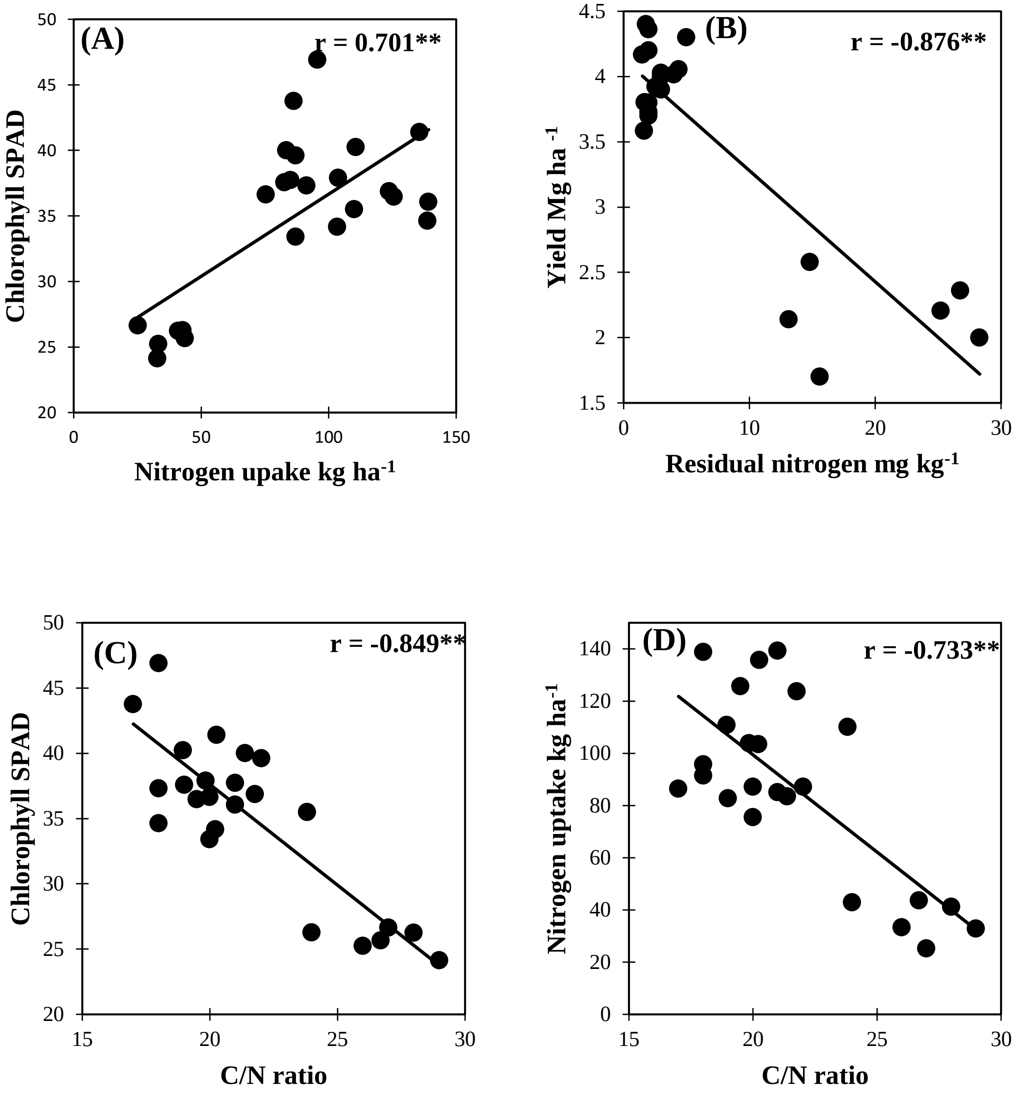

Figure 3.10: Pearson correlation showing significant association between the chlorophyll and nitrogen uptake (A), yield and residual nitrogen (B), chlorophyll and C/N ratio (C), nitrogen uptake and C/N ratio (D) in annual ryegrass when grown in greenhouse under controlled conditions. (*significant at $p \leq 0.05$; **significant at $p \leq 0.01$)

3.3.9.2 Kale

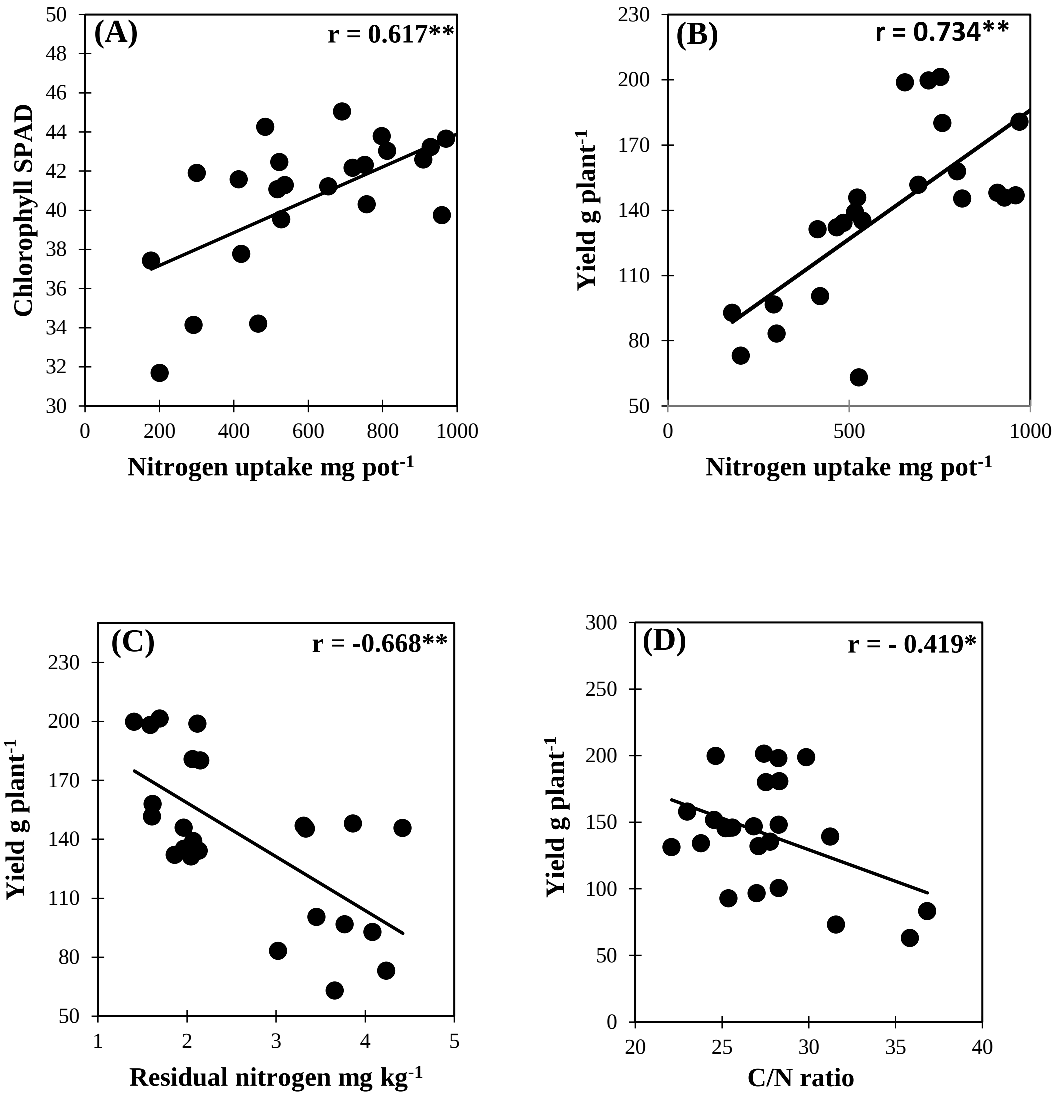

Figure 3.11: Pearson correlation showing significant association between the chlorophyll and nitrogen uptake (A), yield and nitrogen uptake (B), yield and residual nitrogen (C), yield and C/N ratio (D) in kale when grown in greenhouse under controlled conditions. (*significant at $p \leq 0.05$; **significant at $p \leq 0.01$)

3.4 Discussion

3.4.1 Effects of treatments on germination, growth, yield and nitrogen dynamics of annual ryegrass and kale

Seed emergence plays a significant role in the determination of the heathy plant growth and could be affected by certain factors including heavy metal, and high EC or pH concentration in soil amendments. (Steinbrecher & Leubner-Metzger, 2017). Seneviratne et al. (2019) reported that higher concentration of heavy metals negatively affected enzymatic activity of acid phosphatases and protease, involved in seedling establishment. In present study, we observed no significant effects of soil amendments on seed emergence of annual ryegrass and kale (Table 3.2 and 3) which can be attributed to lower heavy metals in WA and SL. Our results are in agreement with the findings of previous studies conducted in different jurisdictions and reported non-significant effects of WA and SL on seed emergence of *Phaseolus vulgaris L.* and *Zea mays* (O'Brien et al., 2002; Wiklund, 2017). Nonsignificant effects of soil amendments on seed emergence might be due lower heavy metals concentration and lower EC in soil and amendments used in this study (Table 2.1).

WA contains significant amount of K^+, that maintains plant internal metabolism and improves plant growth and yield (Füzesi et al., 2015). Melese et al. (2015), reported WA application in an acidic soil increased soil pH and enhanced 41% plant height of *Triticum aestivum* L. compared to control. In present study, we observed similar results in which WA amended treatment showed higher plant height in both crops (Table 3.2 and 3). Similarly, WA application increased number of leaves in kale crop (Table 3.3). Higher plant height and more number of leaves in WA treatment might be due to presence of macro (Ca, Mg, K and P), micro (Mn and

Fe) nutrients and alkaline nature of ash that eventually increased soil pH and improved nutrient uptake (Ochecova et al., 2014; Ribeiro et al., 2010; Salam et al., 2019). However, lower plant height of both crops in SL treatment might be due to high C/N ratio (Table 3.2 and 3) that might have led to N immobilization by soil microorganisms resulted in low mineralization and decreased plant growth. These results are in agreement with the study conducted by O'Brien et al. (2002), who reported N immobilization in soil amended with increased paper sludge.

SL from paper mill has high C/N ratio due to biological process involves in wastewater treatment. Higher C/N ratio leads to N immobilization and results in lower nitrogen uptake. Studies have reported that lower N uptake by the plants results in decreased plant growth including lower chlorophyll content, photosynthesis and yield (Ammary, 2004; Boussadia et al., 2010; Vagstad et al., 2005). In present study, we observed lower chlorophyll in both crops grown in SL treatment (Table 3.2 and 3). These results are in agreement with the study reported by Chrysargyris et al. (2019), who observed SL application decreased chlorophyll in *Calendula officinalis* L.and *Petunia×hybrita* L. SL used in this study exhibited higher C/N (38: 1) ratio caused N immobilization and reduced N uptake in both crops (Table 3.2 and 3). Lower chlorophyll in kale and ryegrass in SL treatment might be due to lower nitrogen release and uptake compared to other soil amendments (Figure: 3.10 A and C, figure: 3.11 A) (Table 3.2 and 3). Ferreira et al. (2016) reported that lower nitrogen concentration in *Scenedesmus dimorphus* leaves lead to decrease in chlorophyll content, reduction in cell growth and plant yield. Additionally, we observed lower photosynthesis rate in SL amended treatment in both crops (Table 3.2 and 3). Kiarostami et al. (2010) reported leaf chlorophyll contents indicates photosynthesis activity, consequently reduction in chlorophyll might have decreased photosynthesis rate and other growth-related parameters (Croft et al., 2017).

Wood ash and SL contains macro/micronutrients that are required by the plant to complete its life cycle (Kinnula et al., 2020; Silva et al., 2019). Application of WA increased soil pH and reduced heavy metal uptake in plants because of high carbon (20-60 %) that make it similar to biochar in nature (Bieser & Thomas, 2019). Dahlin and Stenberg (2017) reported, WA application enhanced K+ tissues concentration and biomass of *Trifolium pratens*e L., cv. Nancy for two consecutive cuts. Similarly, SL as soil amendment increased soil carbon, organic matter, and improved soil physical and chemical properties (Kätterer et al., 2011; Muukkonen et al., 2009; Paustian et al., 2016). In present study, WA, SL and WASL produced 42%, 36% and 42% higher biomass of annual ryegrass as compared to control (Table 3.2). These results are in agreement with the studies conducted by Ziadi et al. (2013) who reported, application of paper mill biosolids increased *Hordeum vulgar*e L. crop yield and enhanced N, P and Ca concentration in *Hordeum vulgare* L. and *Phaseolus vulgaris* L. Increased annual ryegrass biomass in WA as compared to control might be due to availability of essential nutrients, majorly K and Ca, improved plant internal metabolism such as cell division and enlargement and activation of various enzymes that might increased plant growth and yield of annual ryegrass (Ahmadi & Souri, 2019; Guo et al., 2017; Sustr et al., 2019). In present study, WA application increased annual ryegrass and kale yield by 42 % and 28% as compared to control, respectively (Table 3.3). Olugbemi (2019) reported, increased yield of kale and ryegrass by WA application can be attributed to its high solubility, caused rapid mineralization of essential nutrients and more nitrogen uptake (Table 3.2 and 3). In present study, higher nitrogen uptake enhanced yield in kale (Figure 3.11 B). SL treatment exhibited lower kale yield as compared to control (Table 3.3), probably due to high C/N ratio (38: 1) that might have led to increased N immobilization by soil microorganisms caused low N availability and uptake by kale (Figure : 3.11 C and D) (Table 3.3). Our results are in agreement with the findings of previous research conducted by Chrysargyris et al. (2019) and Luo et al. (2018), who reported lower yield of *Calendula officinalis* L. and *Petunia hybrita* L. in SL amended treatments.

Paper mill biosolids increased N mineralization due to their alkaline nature that favours plant N uptake and lower residual nitrogen in soil compared to control (Manirakiza et al., 2019). In addition, paper mill biosolids with higher C/N ratio may increased N immobilization by soil microorganisms (Luo et al., 2018). In present study, we observed more nitrogen uptake by plants and less residual nitrogen in WA treatments contrary to SL treatment. Additionally, increase in N mineralization and higher N uptake by plants in WA treatments might be due to its high solubility and small particle size, makes it more reactive and causes rapid increase in soil pH which stimulated microbial activity resulting in more N mineralization and plant uptake (Bang-andreasen et al., 2017; Cruz-paredes et al., 2017; Silva et al., 2019). We observed lower N uptake and increased residual nitrogen concentration in SL treatments in present study. These results are in line with the studies of Mozaffari and Hays., (2020) who reported, application of SL increased N immobilization and reduced N uptake in *Capsicum annuum* L.,. Increased in N immobilization might be due to higher C/N (38: 1) ratio of the SL treatment compared to control, that might caused N fixation by soil microorganisms, resulted in lower uptake by kale (Figure 3.11 D). Kinnula et al. (2020) reported that application of SL with high C/N ratio (30:1) negatively affected crop yield, reduced N uptake in *Triticum aestivum* and *Avena sativa*.

Additionally, BC amended WA, SL and WASL treatments further enhanced C/N ratio in the soil because of certain volatile fractions that can be readily degradable resulting in lower N uptake in ryegrass (Table 3.2 and 3) (Deenik et al., 2010).

3.4.2 Soil pH and heavy metal concentration in soil and uptake in annual ryegrass and kale

Wood ash and SL consist of high pH due to the presence of carbonates which suggest potential applications as an alternative liming source that can substitute commercial limestone (Royer-Tardif et al., 2019; Simão et al., 2018). Babayemi et al. (2010) reported carbonates and oxides neutralize H^+ and increased OH^- concentration in the soil solution, resulting in increased soil pH. Additionally, WA reacts rapidly due to its small particle size, causes solubilization of H^+ that present at exchangeable site, into the soil solution and could enhance soil pH (Hu et al., 2020; Kapembwa et al., 2020). In the present study, we observed that WA and SL alone and combine applications with BC increased soil pH from 5.2 to target pH of 6.2-6.3 (Table 3.4 and 6). These results are in agreement with the studies conducted by Manirakiza et al. (2019) who reported, application of paper mill biosolids alone and in combination with BC increased soil pH compared to commercial limestone (Adotey et al., 2018).

There is a potential risk of heavy metals (Cr, Ni, As, Cu, Pb and Cd) contamination in WA and SL due to utilization of used oil during the combustion process (Cherian & Siddiqua, 2019; Sharifi et al., 2013; St. Luce et al., 2017). Heavy metals are non-degradable elements that might accumulate in plant tissues and directly or indirectly could have negative effects on animals and human health through contaminated food and feed (Augusto et al., 2008; Pateriya et al., 2021).

Though heavy metals concentration in WA and SL used in this study were within the allowable limits (Table 2.1). In present study, we observed that SL did not affect Cr and Ni concentration in soil used for annual ryegrass cultivation although higher concentration of Pb and Cd was observed in SL than control (Table 3.4). Higher concentration of Pb and Cd in SL might be due to higher application rate (94 Mg ha^{-1}) compared to WA (20 Mg ha^{-1}). Additionally, Gagnon and Ziadi (2021) reported that mineralization of the paper mill biosolid over time may gradually release heavy metals in bioavailable form. In the present study, BC application with soil WA and SL significantly increased Ni, Cu, As and Cd concentration in soil used for annual ryegrass cultivation and these results are in line with the studies conducted by Yousaf et al. (2017) who reported that application of wood derived BC reduced heavy metal concentration. Absorption of heavy metals in the soil via BC might be due to its large surface area and higher cation exchange capacity (Lu et al., 2012). However, in present study, heavy metal concentration in all treatments of annual ryegrass soil, amended with paper mill waste were below the allowable limit for Cr, Ni As, Pb and Cd (64, 50, 12, 70 and 1.4 mgkg^{-1}) established by Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007). Whereas in annual ryegrass shoots, we observed nonsignificant effects of soil amendments on heavy metal concentration except As (Table 3.5). WASL showed higher As (0.9 mg kg^{-1}) concentration compared to control (Table 3.5) that might be due to higher amendment concentration of WA + SL together, however, As concentration was below the allowable limit (2 mg kg^{-1}) (Rodr et al., 2019) (Table 3.5). Lower concentration of heavy metals in annual ryegrass shoots (Table 3.5) might be due to lower concentration of heavy metals in the respective amendments (Table 2.1). Chu et al. (2018) reported higher heavy metals (Cr, Cd and Pb) accumulation in *Lolium perenne* L. roots than shoots. Waterlot et al. (2019) reported, annul ryegrass posses a dense root system and could store heavy metals in roots by making organic complexes with the heavy metals. Similarly, in this study, metalloids concentration in shoots of annual ryegrass were below the permissible limit for foraging (As, 2 mg kg^{-1} and Pb , 10 mg kg^{-1}) (Rodr et al., 2019). Similarly, Cr (5-100 mg kg^{-1}), Ni (10 mg kg^{-1}), and Cd (1 mg kg^{-1}) level were below the allowable limit that can be toxic for annual ryegrass growth (Oliveira, 2012; Osmani et al., 2015).

In present study, we observed soil used for kale cultivation contains higher Cr, Ni, Cu, Pb and Cd concentration in SL treatment compared to control (Table 3.6). Manirakiza et al. (2019) also confirmed that paper mill biosolids increased heavy metals concentration that might be due to higher application rate that causes more heavy metal addition in soil than the conventional limestone, similar to results reported in present study (Table 2.1). Liang et al. (2017), reported application of SL increased Cu concentration in soil due to high extractable water content and organic carbon. Additionally, Wierzbowska et al. (2018), reported increased mineralization of the organic matter in waste substances enhanced bioavailability of heavy metals in the soil. Despite of increased heavy metal concentration in kale soil compared to control, overall heavy metal concentration was lower in kale soil than the permissible limit of Cr, Ni As, Pb and Cd (64, 50, 12, 70 and 1.4 mg kg^{-1}, respectively) established by Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007). In kale shoots, we observed higher concentration of Cr, As and Pb in WASL treatment compared to control (Table 3.7), such increased in heavy metal concentration might be due to 80 % WA (16 Mg ha^{-1}) and 20 % SL (18 Mg ha^{-1}) added more heavy metals in soil compared to Control. Manzoor et al. (2018) reported that vegetables e.g., kale known as hyperaccumulator plant due to its efficient root system with can uptake, translocate and store heavy metals from the soil even at very low concentration. Therefore, in present study, treatments with mill amendments showed higher concentration of Cr, Pb and Cd shoots in compared to control, however, heavy metals concentration in kale shoots was below the permissible (Cd 0.2 mg kg^{-1}, Pb 2 mg kg^{-1}, Ni 10 mg kg^{-1}, As 2 mg kg^{-1}, Cr 1.30 mg kg^{-1}) limits set for plant production (WHO 1996).

In present study, co-application of BC along with WA, SL and WASL in kale soil, enhanced Cr, Pb and Cd concentration in soil and reduced their uptake in kale shoots. These results are in conformity with the study done by Yousaf et al. (2017) who reported that application of pinewood biochar (at 2% w/w) reduced uptake of Cr, Ni and Pb by 76 %, 68 % and 86 % in *Triticum aestivum*. Reduced uptake of heavy metals uptake with BC application might be due to large surface area of BC particles, presence of groves (Figure 2.12) and higher cation exchange capacity (Liu & Zhang., 2012). In addition, BC used in this study was produced from yellow pinewood feed stock, pyrolyzed at 550°C with high CEC, and recalcitrant carbon (Table 2.3) and may contain functional groups that might have increased ability of BC to adsorb heavy metals in the soil (Chen et al., 2018; Yang et al., 2019). In addition, increased in soil pH might also help BC in the adsorption of heavy metals. Deng et al. (2017) reported, increased soil pH causes deprotonation and made BC functional groups to coordinate with metal ions that lead to greater adsorption ability. Results from present study are in line with the previous studies in which BC addition enhanced heavy metal adsorption and reduced uptake in shoot tissues of plants (Lin et al., 2020; Ma et al., 2021; Sun et al., 2021).

3.5 Conclusion

The aim of this study was to assess the potential of WA, SL WASL alone and in combination with BC as alternative liming material and nutrients as well as to evaluate their effects on growth, yield, nitrogen dynamics and heavy metals mobility in soil-plant system of annual ryegrass and kale. The study results indicated that WA and its combination with SL increased plant growth and physiological parameters (plant height, chlorophyll and photosynthesis), yield and nitrogen uptake in annual ryegrass and kale due to presence of essential nutrients. Additionally, WA could potentially be used in agriculture production as a suitable soil amendment due to very good levels of K, P and Ca and improved soil chemical and biological properties (Pukalchik et al., 2017; Wójcik et al., 2020). However, higher C/N ratio in SL decreased plant growth parameters (chlorophyll and photosynthesis and N uptake) in annual ryegrass and kale. Furthermore, heavy metal concentration in biosolids amendments with different formulations observed were within the CCME limits (CCME, 2007) (Table 2.1). Although paper mill amendments contained low heavy metal concentration; BC addition further decreased heavy metal concentration in plant tissues by adsorbing them in the soil. Based on the results, it can be concluded that application of WA and WASL could be used as liming and nutrient source in podzolic soils., In this study, SL showed higher C/N ratio that cause lower N mineralization and uptake. However, there is need to develop techniques to lower down the C/N ratio of the sludge or more N fertilizer can be utilized along with SL application that could be helpful in its application for agriculture production. Additionally, low heavy metal concentration in mill-based amendments indicates utilization of these amendments in agriculture production systems.

3.6 References

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Chapter 4

General discussion and conclusion

The objectives of the current study were:

1. To investigate the effects of WA, SL alone, and in combination with BC on seedling emergence, growth and yield of annual ryegrass and kale.
2. To determine the effects of WA, SL alone and in combination with BC on soil pH, N uptake and heavy metals in soil-plant systems of annual ryegrass and kale

These objectives were accomplished by conducting two greenhouse experiments, including two different soils, as mentioned in Chapters 2 and 3. From these two studies we have assessed utilization of WA, SL and their combination WASL with and without addition of BC as a suitable liming and nutrient source in two different soil types (Table 2.1 and 3.1). In study 1 (Chapter 2), we used soil with pH of 5.7 and 2.7 % organic matter. In study 2 (Chapter 3) soil was used with pH 5.2 and 4.5% organic matter. In both studies we observed that WA and WASL produced higher forage biomass of annual ryegrass and higher yield of kale compared to control treatment. Overall, higher yield of both crops was observed in study 2 as compared to study 1. Generally, in both studies mill amendments showed a similar trend e.g., produced more yield than control and increased soil pH from the initial soil pH status and maintained till harvest of the crops.

Overall, we observed that WA application increased plant growth, enhanced nitrogen uptake, and increased crop yield of annual ryegrass and kale in both studies (chapter 2 and 3). In addition, treatments amended with WA subsequently showed lower residual nitrogen that might be due to higher N uptake in the treatments amended with WA. On the other hands SL application have not produced promising results and showed lower plant growth including plant height, photosynthesis and nitrogen uptake that eventually contributed to lower biomass yield in annual ryegrass and kale. This lower biomass yield can be attributed due to higher C/N ratio of SL that might have caused nitrogen immobilization in the soil. These results are in agreement with study done by Sippola et al. (2003), who reported that lower nitrogen uptake by plants amended with paper mill sludge with higher C/N ratio resulted in N immobilization.

Previous studies conducted by Gagnon and Ziadi, (2012) and Manirakiza et al. (2020) observed that paper mill biosolids (WA and SL) showed increased soil pH. In present study, we also observed similar results in which WA and SL application significantly increased soil pH from 5.7 and 5.2 to target soil pH of 6.3 in both studies (chapter 2 and 3). However, amount of SL added was multifold compared to WA, which had changed C/N ratio, N availability in soil and consequently in plant uptake. These findings are quite in line with the study conducted by Sharifi et al. (2013), who reported an increased soil pH with WA application and might be due to the higher calcium carbonate content of ash and nutrient content.

Before conducting this study, heavy metals in mill amendments (WA, SL and WASL) was a major concern and therefore analyzed before application. We observed lower heavy metal concentration in analysis reports of WA and SL composite samples, and were below CCME limit for biosolids application (Table 2.1). Furthermore, we observed that WA, SL, and WASL amendments did not exceed heavy metal concentration in annual ryegrass and kale soils. Heavy metal concentrations observed in soil of these studies were below the allowable limit (64, 50, 12, 70 and 1.4 mg kg^{-1}, in Cr, Ni, As, Pb and Cd respectively) established by Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health (CCME, 2007). In present studies, heavy metal concentration in shoot tissues of annual ryegrass were below the limits for forages (As 2 mg kg^{-1} and Pb 10 mg kg^{-1}) (Rodr et al., 2019). Whereas Cr level was below 5-100 mg kg^{-1} (Oliveira, 2012), and Ni (10 mg kg^{-1}) and Cd (1 mg kg^{-1}) were below the allowable limit (Osmani et al., 2015). Similarly, in kale shoot tissues, heavy metals concentrations were within the permissible limits set for vegetable production (0.2, 2, 10, 2, and 1.30 mg kg^{-1} for Cd, Pb, Ni, As, and Cr, respectively) (WHO, 1996). BC amendment with WA, SL and WASL further reduced heavy metals uptake in shoot tissues of both crops. Reduction in heavy metals uptake in plant tissues can be attributed due to: (1) heavy metal exchange with cations, e.g., Ca^{2+} (2) formation of different complexes with functional groups and complexation with the free hydroxyl of mineral oxides (3) surface precipitation and physical adsorption and other attributes including higher cation exchange capacity (CEC), and large surface area of BC particle (Liu et al., 2017; X. H. Liu & Zhang, 2012; Whitty & Tollervey, 2013; Yousaf et al., 2017; Yuan et al., 2011). These results indicate the mill amendments could be used with BC to minimize the heavy metal uptake in plants.

4.1 Conclusion and recommendation

Application of WA and WASL as soil amendments enhnaced plant growth and yield in both soils. In addition, these amendments improved soil pH and showed better performance in podzolic soils when compared with limestone application. In the present studies, we have used two soils with different pH (5.7 and 5.2). WA application in soil with pH 5.7, increased soil pH 5.7 to target pH 6.3 and produced 71% higher biomass of annual ryegrass and 28 % higher yield in kale than control (chapter 2). Similarly, in second study with soil pH 5.2, WA produced 42% higher biomass of annual ryegrass and 28 % more yield in kale than control and increased initial soil pH 5.2 to the target pH 6.3 (chapter 3). Based on the results of these two studies, it can be concluded that:

i. WA was efficient in achieving and maintaining the target pH (6.3) in both soils till the harvest of both crops, suggesting that it can be used as a liming material.
ii. Significantly increased nitrogen uptake and lower residual soil nitrogen was observed in WA, indicating better soil amendment with improved NUE in both crops and soils.
iii. WA and WASL treatments showed superior agronomic performance and produced higher yield in annual ryegrass and kale compared to control and other treatments.
iv. Paper mill amendments (WA, SL and WASL) application in annual ryegrass and kale soil showed lower heavy metal concentration and were within the allowable limit established by Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health for Cr, Ni As, Pb and Cd (64, 50, 12, 70 and 1.4 mg kg^{-1}) though heavy metals in soil was comparatively higher than in mill amendments.
v. Annual ryegrass showed lower heavy metal uptake than kale due to its excluder root type system which act as barrier for heavy metals uptake.
vi. Biochar addition reduced heavy metal uptake in plant tissues and retained them in the soil.

vii. Results from this study indicate that paper mill amendments (WA, SL and WASL) contain lower heavy metal concertation than the permissible limits and could be used as liming and nutrient source for annual ryegrass and kale without toxic effects on growth and forage production. However, BC addition further reduced heavy metals uptake, which indicates the combination of amendments with BC can minimize heavy metal uptake risk in forage and vegetable crops.

Further studies exploring the potential of mill amendments (WA, SL and WASL) as liming and nutrient source needs to be done under field conditions. These amendments may alter soil microbial community structure and abundance, soil mineralizable nitrogen, soil carbon pools and overall soil quality and health. In addition, from current studies, we have identified that WA has the potential to be used as liming and nutrient source for crop production and could be an alternate soil amendment and liming material compared to standard limestone. Newfoundland and Labrador government is leasing 64,000 hectares to new and existing farmers and entrepreneurs to enhance food production in the province. Mostly, these soils will be newly clear forest lands with low pH. Use of WA as soil amendment on these newly cleared forest land will rais soil pH, improve fertility and physiochemical properties. This practice will be beneficial to amend low pH and poorly fertile soils as well as significantly reduce land disposal cost for the paper mill industry. However, addition of optimum rates should be determined before WA application to minimize the heavy metal risk, or these soils should be assessed each year before and after WA application to check heavy metal concentrations are within safety limits.

4.2 References

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