The Hydrochar Physiochemical Properties Following Different Aging Methods, and Its Impact on Kale Seed Germination, Plant Growth and Nutrient Elements Accumulation

by

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Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

DEDICATION PAGE

To my grandfather and grandmother, in loving memory.

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ABSTRACT

Hydrochar, a product from hydrothermal carbonization (HTC) of biomass waste, has gained considerable attention in agriculture as a potential soil amendment. However, it has been found to exhibit phytotoxicity, which could inhibit plant growth. This study aimed to investigate the effects of three modification methods (water washing, microbial aging, and freezing-thawing aging) on the physicochemical properties of hydrochar derived from coffee grounds. Furthermore, the study evaluated the effects of applying four types of hydrochar with 10% and 20% application rates in growing medium on seed germination and plant growth. The results revealed that the physiochemical properties of hydrochar were significantly ($P<0.05$) altered by the different hydrochar pre-treatment methods. Moreover, the seed germination experiment showed that microbial aged hydrochar (MHC), water-washed hydrochar (WHC) and freezing-thawing aged hydrochar (FTHC) were effective in promoting germination compared to fresh hydrocar (FHC). The plant growth experiment demonstrated that 10% MHC was the most effective treatment in mitigating plant growth inhibition, despite some inhibitory effects still being observable compared to the control. Further studies should focus on a large-scale field study and investigate the effect of improved hydrochar on soil biology.

Keywords: hydrothermal carbonization (HTC), hydrochar, aging, kale, plant growth, soil amendment

LIST OF ABBREVIATIONS USED

%	Percentage
AM	Arbuscular Mycorrhizal
BET	Brunauer Emmett Teller
℃	Degrees Celsius
CEC	Cation Exchange Capacity
cm	Centimeter
DI	Deionized
EC	Electrical Conductivity
EDS	Energy Dispersive X-Ray Spectroscopy
FHC	Fresh Hydrochar
FTHC	Freezing Thawing Aged Hydrochar
FTIR	Fourier Transform Infrared
g	Gram
GE	Germination Energy
GI	Germination Index
GR	Germination Rate
HMF	Hydroxymethylfurfural
HTC	Hydrothermal Carbonization
HTG	Hydrothermal Gasification
HTL	Hydrothermal Liquefaction
L	Liter
MGT	Mean Germination Time
MHC	Microbial Aged Hydrochar
PAH	Polyaromatic Hydrocarbons
SEM	Scanning Electron Microscope
SPAD	Soil Plant Analyses Development
SVI	Seed Vigour Index
TDS	Total Dissolved Solid
WHC	Water Washed Hydrochar

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Eventually, I have completed my MSc project. I thought I would be happy or even excited, but as I write this acknowledgements part, I am filled with a sense of calm satisfaction. Looking back my time at Dal AC, memories flood my mind like raindrops tapping on windshield.

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Chapter 1 Introduction

1.1 General introduction

Soil is an invaluable and irreplaceable natural resource that plays a vital role in agricultural and food production (Rickson et al., 2015). However, the rapid population growth and a limited amount of arable land have increasingly put pressure on agriculture ecosystem to meet the growing demand for food (Wang, 2022). The pursuit of higher agricultural productivity has also led to the use of inappropriate agricultural practices such as the overuse of chemical fertilizers and pesticides, as well as over farming (Zhang and Wang, 2020). As a result, soil degradation, soil contamination, and soil nutrient deficencies are intensifying and becoming more prevalent, which would trigger vicious spirals in food production and food security (Rahut et al., 2022). In response to these challenges, hydrochar derived from biomass has recently attracted attention as a soil amendment with the potential to effectively address these issues.

Alternatively, modern agriculture with a focus on sustainability faces interconnected challenges. Biomass waste production worldwide was estimated at 100 billion metric tons per year (Cho et al., 2020). Sources of biomass waste include agricultural waste, food processing waste, and forestry residuals (Kaygusuz and Türker, 2002; Yu et al., 2021). Typically, the biomass wastes are disposed of in landfills or go to incineration, which would result in environmental concerns (air pollution, accelerated climate change) and economic costs (Cho et al., 2020; Grewal et al., 2018). Consequently, the conversion of biomass waste into hydrochar with additional value as a soil amendment, represents an ideal solution for sustainable agriculture.

Hydrochar is a carbon rich solid material that can be made from hydrothermal carbonization (HTC) or hydrothermal liquidfication (HTL) (Zhang et al., 2019). The concept of using hydrochar to improve soil properties is not new, its roots can be traced back to Terra Preta, an extremely fertile soil type typically found in the central Amazonian regions, where agricultural yields are considerably higher than those in the surrounding soil (Kambo and Dutta, 2015; Lan et al., 2021). The accumulated stable

carbonized components in the soils of Terra preta were thought to have contributed to the soil's productivity (Islam et al., 2021). Considering the high fertility of Terra Preta soils, researchers subsequently investigated the practicality of using hydrochar in modern agriculture.

Hydrochar can be used in multiple applications, such as soil amendment, water treatment, and energy production (Azzaz et al., 2020; Berge et al., 2015; Islam et al., 2021; Kambo and Dutta, 2015). It is well known that the porous structure of hydrochar can reduce bulk density of soil and increase soil porosity and water retention (Kalderis et al., 2019; Mau et al., 2020). Some studies have demonstrated the potential of hydrochar application to increase crop yields (Baronti et al., 2017; Rillig et al., 2010). However, other research also indicated that hydrochar inhibits plant growth due to inherent phytotoxicity (Bargmann et al., 2013; Busch et al., 2013; Roehrdanz et al., 2019). Currently, a number of studies have been carried out using various methods, i.e., acidification, aging and pyrolysis, to eliminate phytotoxicity in hydrochar, making it suitable for agricultural application (Hitzl et al., 2018; Mumme et al., 2015; Yu et al., 2019).

In this study, research focus was on evaluating the suitability of hydrochar as a soil amendment in agricultureal fields. We used coffee (*Coffee arabica*) grounds as feedstock to produce hydrochar, applied three pre-treatment methods, i.e., water washing, microbial aging, and freezing-thawing aging to modify the properties of hydrochar and test their efficacy for plant growth. Currently, very few studies have investigated the effect of coffee grounds based hydrochar on the growth of kale (*Brassica oleracea* L. var. acephala DC.). Neither the effects of microbial aging nor freeze-thawing aging of hydrochar have been tested on kale growth. Therefore, it is necessary to conduct such a study to investigate alteration in properties of spent coffee grounds hydrochar subject to different aging methods and the effect of modified hydrochar on seed germination, plant growth and nutrient elements accumulation.

1.2 Objectives

The overall goal of this study was to evaluate the impact of aged-hydrochar on the growth of kale plants.

The specific objectives were:

1. To investigate the change in physical and chemical characteristics of hydrochar under different pre-treating methods.
2. To investigate the effects of the different modified hydrochars on kale seed germination indices, plant growth and chemical quality.
3. To identify the most suitable application rate of hydrochar for the production of potted kale plants.

The hypotheses of this project were:

1. Aging will improve the properties of hydrochar relevant to plant growth.
2. Aged hydrochar will reduce phytotoxicity and will promote seeds germination and plant growth.
3. 20% will be the optimal application rate for promoting the growth of kale plants.

1.3 Organization of thesis

This thesis consists of six chapters. Chapter 1 serves as an introduction and includes an overview of the project, the thesis objectives and hypothesis, and the organization of the thesis. Chapter 2 is dedicated to a literature review that provided a summary of hydrochar production, hydrochar reaction mechanisms, the application of hydrochar in agriculture, and methods for reducing hydrochar phytotoxicity. Chapter 3 presents original research that investigated the impact of three pre-treatment methods on the physiochemical properties of hydrochar and on seed germination indices. Chapter 4 presents original research that explored the growth response of kale under three modified hydrochars and a control at different application rates. Chapter 5 is the recommendation for future work. Finally, Chapter 6 is the overall conclusion of this study. Chapter 3 has been published to the MDPI Horticulture. Additionally, a manuscript based on Chapter 4 is currently being planned for submission to the MDPI Plants.

Chapter 2 Literature Review

This review summarizes hydrochar production, reaction mechanisms, and recent updates on hydrochar applications in agriculture, followed by methods to improve the characteristics of hydrochar and future research prospects as soil amendment.

2.1 Production of Hydrochar

2.1.1 Feedstock for Hydrochar Production

The production of hydrochar starts with the selection of feedstocks. Based on recent studies, a wide variety of feedstocks have been used to produce hydrochar. As far as the source of feedstocks is concerned, which could be classified into different categories, namely, energy crop (e.g., miscanthus and switchgrass) (Smith et al., 2016), agro-forestry residue (Cheng et al., 2021; Kalderis et al., 2019; Wang et al., 2022), sludges (He et al., 2013; Liu et al., 2021; Ren et al., 2017), animal manures (Fu et al., 2022; Lang et al., 2019; Li et al., 2021; Xiong et al., 2019), food wastes (Berge et al., 2015; Saqib et al., 2018; Sharma and Dubey, 2020), algal residues (Du et al., 2012; Masoumi and Dalai, 2020; Toptas Tag et al., 2018), aquaculture wastes (Kannan et al., 2017, Kannan et al., 2018), and municipal solid waste (Lin et al., 2017; Śliz et al., 2022; Wei et al., 2017). Apparently, the majority of feedstocks possess very low value or has been viewed as waste material. However, converting biomass wastes via hydrothermal carbonization (HTC) into value-added products does not only reduce pollution but also, conserves resources (Fan et al., 2018; Lin et al., 2017).

2.1.2 Hydrochar production process

HTC is a promising thermochemical process that converts biomass into a coal-like solid product (hydrochar) under high temperature and pressure conditions (Wang et al., 2018). Alternatively, HTC is a fast artificial process that mimics the natural process of biomass coalification (Heidari et al., 2019). In general, the HTC process reaction time range is 5–240 min (Kambo and Dutta, 2015), or even up to 12 h (Ulbrich et al., 2017) or 48 h (Wilk

et al., 2021). The HTC reaction pressure reflects the vapour pressure of water in a confined system when heated, with a range of 2–10 MPa (Wang et al., 2019). The reaction temperature of HTC ranges between 180 and 260 °C (Kambo and Dutta, 2015). To date, numerous studies have explored and documented various HTC production processes (Afolabi et al., 2020; Chen et al., 2017; Ma et al., 2019; Yang et al., 2018); thus, this review will not dwell on this point. The process flow of HTC is shown in Fig. 2.1.

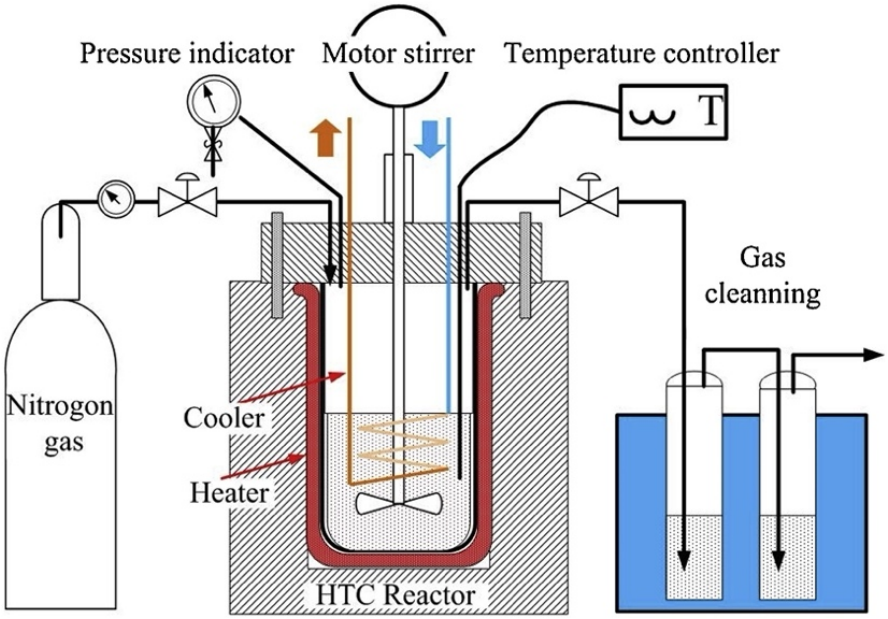


Fig. 2.1. The overall HTC production process (Ma et al., 2019).

HTC operational conditions (e.g., retention time, reaction temperature) can affect the characteristics of the resulting hydrochar (Yan et al., 2010). Wang et al. (2018) observed that, with an increase in the HTC retention time, the severity of the HTC reaction also increased. Severe HTC conditions promote the dissolution of elemental components of hydrochar into the aqueous phase. Furthermore, small fragments dissolved in the liquid phase could increase hydrochar yield by polymerization (Nakason et al., 2018; Wang et al., 2018). Chen et al. (2020) found that an increase in the HTC retention time from 1 to 5 hours resulted in an increase in the content of ash in hydrochar while decreasing the contents of N, S, and O. In a study conducted by Romero-Anaya et al. (2014), the authors investigated the effect of retention time (12, 24 and 48 hours) on the morphology of hydrochar. They discovered that carbon microspheres formed more effectively in

hydrochar after a 24-hour retention period than after a 12-hour retention period. Additionally, the hydrochar yield is barely affected by the extension of HTC retention time (Nakason et al., 2018). However, according to the study by Gu et al. (2018), the yield of hydrochar produced from fallen leaves and iron sludge decreased from 64.04% to 47.82% when the retention time was extended from 1 hour to 5 hours. Also, Jaruwat et al. (2018) suggested that the hydrochar yield fluctuated with the retention time. They observed a decrease in hydrochar yield during the first 12 hours of the retention period, which was followed by a rise as the retention period was extended to 25 hours. The relationship between hydrochar yield and retention time is complex, which needs more further studies to explore the effect of retention time on hydrochar yield. Reaction temperature is another crucial factor that influences the final properties and distributions of hydrochar products (Chen et al., 2020; Gu et al., 2018; Romero-Anaya et al., 2014). Mäkelä et al. (2015) investigated the effect of various HTC process conditions (180°–260 °C, 1–6.25 hours) on hydrochar properties and found that reaction temperature was 3–7 times more influential than retention time. Gao et al. (2016) discovered that the yield of hydrochar decreased from 46.4% to 40% as the temperature increased from 220° to 300 °C. The decreasing trend was also confirmed by Afolabi et al. (2020). This decrease in yield can be attributed to biomass degradation at high temperatures (Sabio et al., 2016). However, it is also important to note that higher HTC reaction temperatures tend to increase energy consumption, which leads to an increase in cost.

The main products of HTC are solid hydrochar, process water, and gas (CO_2) (Pauline and Joseph, 2020). As water serves as the reaction medium, biomass does not need to be pre-dried for the HTC process, making it more energy-saved and environmentally efficient (Yu et al., 2019). The HTC method is also less energy-intensive than the pyrolysis process that produces biochar since it operates at a lower temperature (around 200°–300 °C) (Güleç et al., 2022; Zhang et al., 2019). Moreover, HTC generates high yields while producing less polluting air emissions (Bardhan et al., 2021). In addition, Kambo and Dutta (2015) proposed that HTC can be further turned into hydrothermal liquefaction (HTL) and hydrothermal gasification (HTG) at higher temperatures (over 260 °C). The main output of the HTL process is biocrude oil (Khan et al., 2021), whereas

the main product of the HTG process is gaseous products such as CH_4, H_2, CO, CO_2, and C_1–C_4 carbon gases (Pavlovič et al., 2013). A comparison of the process conditions for HTC, HTL, and HTG is shown in Fig. 2.2. Since the properties and potential applications of hydrochar products are the focus of this review, further discussion of HTL and HTG will not be provided in this context.

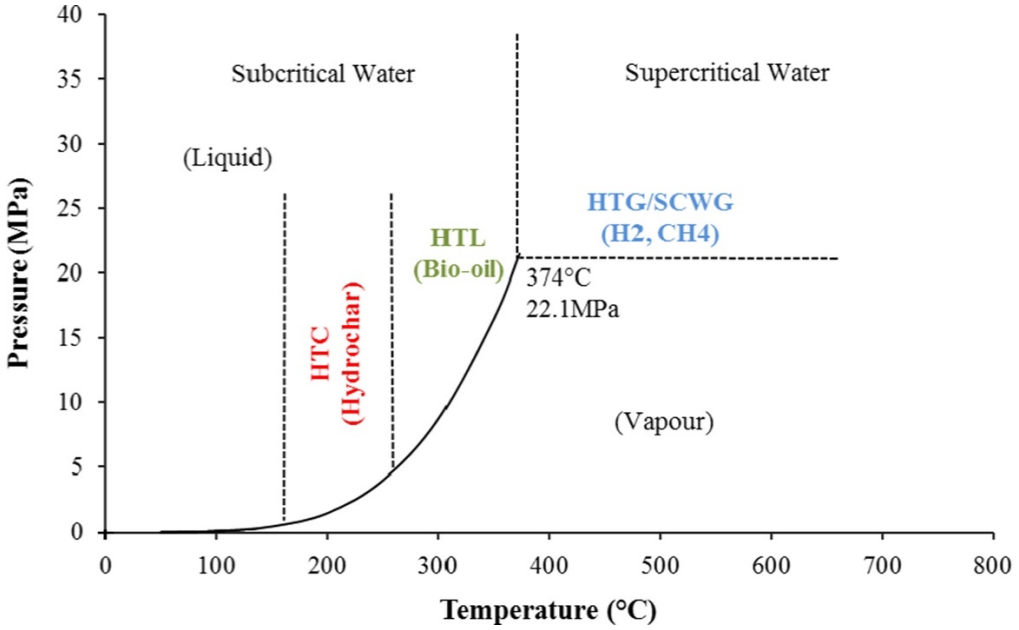


Fig. 2.2. The different hydrothermal process conditions of HTC, HTL and HTG (Kambo and Dutta, 2015).

2.1.3 HTC reaction mechanisms

Hydrochar is generally produced by subjecting biomass to a series of complex reactions at elevated temperatures and pressures. In recent years, many studies have been carried out to better understand the hydrochar formation mechanisms (Islam et al., 2021; Khan et al., 2021; Pauline and Joseph, 2020; Wang et al., 2018). The main reaction pathways for the HTC process include dehydration, hydrolysis, decarboxylation, polycondensation, and aromatization (Nawaz and Kumar, 2022). The following offers a detailed explanation of the mechanism of the HTC reaction.

Lignocellulose consists of three polymers: cellulose, hemicellulose, and lignin (Zhang et al., 2011). In the HTC process, the water used as the reaction medium is kept in a subcritical state at elevated temperature and pressure, under these conditions, water acts as both a reactant and a catalyst, which lowers the polymer's activation energy level and helps speed up the hydrolysis of the biomass (Kambo and Dutta, 2015). Cellulose hydrolysis occurs at an approximate temperature of 230 °C (Reza et al., 2014). Cellulose is decomposed into oligomers, and these oligomers are subsequently broken down into glucose and fructose (Sevilla and Fuertes, 2009). Following a series of dehydration, cleavage, and isomerization processes, the hydrolysis products yield intermediates such as 5-hydroxymethylfurfural (5-HMF) and furfural (Wang et al., 2018; Zhang et al., 2019). The intermediates and other derivatives obtained undergo further polymerization, reverse aldol condensation, and intermolecular dehydration. Additionally, these intermediates lead to the formation of acrylic, lactic, acetic, and formic acids during their disintegration; these organic acids accelerate degradation by decreasing the pH and acting as catalysts. Intermolecular dehydration accelerates aromatization when the concentration of aromatic clusters in an aqueous solution reaches a saturation level, resulting in burst nucleation. By diffusing outward, the nuclei form reactive oxygen functional groups such as ethers and quinine (Sevilla and Fuertes, 2009). In contrast, the hydrolysis of hemicellulose takes place at lower temperature, around 180 °C (Fernández-Sanromán et al., 2021). The hydrolysis of hemicellulose produces xylose, which is then subsequently dehydrated to form the intermediate furfural. Finally, the intermediate furfural obtained results in the formation of carbon microspheres via polymerization (Kang et al., 2012). Compared to cellulose and hemicellulose, lignin undergoes hydrolysis at relatively high temperature, approximately 260 °C (Reza et al., 2014). Wahyudiono et al. (2007) also suggested that the hydrolysis of lignin occurs at temperatures exceeding 200 °C. Dissolved lignin undergoes a series of hydrolysis, dealkylation, and polymerization reactions, ultimately resulting in the formation of phenolic char, while undissolved lignin undergoes solid-to-solid conversion, polyaromatic char is formed (Fang et al., 2008).

Non-lignocellulosic materials, such as proteins and lipids, exhibit different hydrochar formation mechanisms compared to lignocellulosic materials. Proteins undergo

hydrolysis to yield polypeptides, which subsequently hydrolyze further to form oligomers such as tetramers, trimers, and dimers (Pauline and Joseph, 2020). Dimers consist of both linear polypeptides and cyclic polypeptides, which are capable of interconverting. Specifically, cyclic polypeptides can be hydrolyzed to produce linear polypeptides, while linear polypeptides can be transformed into cyclic polypeptides via cyclodehydration (Wang et al., 2022). With further hydrolysis, oligomers are broken down to form amino acids, which can be classified into five categories: (1) aromatic/heterocyclic amino acids – histidine, proline, tyrosine, and phenylalanine; (2) alkyl amino acids – valine, alanine, leucine, isoleucine, and glycine; (3) carboxylic amino acids – glutamic and aspartic acid; (4) hydroxyl amino acids – threonine and serine; and (5) amino acids with amino groups in R position – arginine and lysine (Zhuang et al., 2019). In the hydrothermal process, the main reactions involving amino acids are decarboxylation and deamination (Wang et al., 2022). Glycine, phenylalanine, and glutamic acid can be converted into CO_2 and amines by decarboxylation, and aspartic acid and alanine undergo deamination to form malic acid and lactic acid (Zhuang et al., 2019). Nitrogen-containing ring compounds are the final products of the Maillard reaction between amines and sugars or carbonyl-containing sugars. This reaction also produces aldehydes, furans, pyrroles, pyridines, and pyrazines (Wang et al., 2018).Additionally, the Maillard reaction has been found to be related to the HTC process temperature, in a research carried out by Danso-Boateng et al. (2015), they observed that Maillard reaction products were not observed when the reaction temperatures below 180℃. However, when the temperature was increased to over 180℃, the Maillard reaction was observed to be dominant. For lipids, lipids can decompose into fatty acids and glycerol under high temperature and pressure conditions during HTC. With further dehydration and decarboxylation, fatty acids break down into hydrocarbon compounds (Watanabe et al., 2006). With the increase of reaction temperature, the oxygen-containing functional groups of aliphatic methylene groups on the aromatic or heterocyclic rings undergo further thermal decomposition, resulting smaller molecules. These smaller molecules then undergo aromatization and ring condensation to generate hydrochar (Wei et al., 2018).

2.2 Effect of Hydrochar on Soil Properties

2.2.1 Effect of hydrochar on soil physical properties

Many researchers have investigated the effect of hydrochar on soil physical properties (George et al., 2012; Kalderis et al., 2019; Mau et al., 2020). In the study carried out by Mau et al. (2020), it was found that an application of 2% hydrochar produced from poultry litter as soil amendment resulted in decreased bulk density. Also, in a study conducted by Abel et al. (2013), they discovered that the addition of maize silage hydrochar to soil increased soil porosity from 6.3% to 11.5%. Álvarez et al. (2017) reported that the combination of peat and hydrochar as growing media increased soil water-holding capacity by 21% compared to the application of peat alone. However, Kalderis et al. (2019) demonstrated that adding 5% orange peel hydrochar to soil had no significant effect on the water-holding capacity of soil; with further increase in the application rate to 10%, soil water-holding capacity reduced by 7% and as the application rate was increased to 15%, soil water-holding capacity decreased by 15%. As reported by Abel et al. (2013) and Röhrdanz et al. (2016), the effect of hydrochar addition on soil water-holding capacity was dependent upon soil type, with sandy soils being more affected than clay-rich soils. Additionally, the impact of hydrochar on soil properties could be influenced by the raw material used and the HTC process conditions, as different raw materials and production parameters may affect the final properties of the hydrochar (Sun et al., 2014). Mau et al. (2020) verified similar results and reported that addition of hydrochar produced at 250 °C to soil resulted in a smaller soil porosity compared to the hydrochar produced at 200 °C.

2.22 Effect of hydrochar on soil chemical properties

Soil pH is an important indicator of soil acidity and soil health. Sun et al. (2020) observed that with addition of 1.5% wood dust hydrochar reduced the pH of soil from 6.91 to 6.07. A possible explanation for the decrease could be the presence of organic acids on the surface of hydrochar (Saha et al., 2019). Rillig et al. (2010) noticed that applying 20%

hydrochar obtained from beetroot chips raised the soil pH level from 7.2 to 7.6. The authors attributed the pH rise to microbial reduction processes. Currently, little research has been conducted to investigate the long-term impact of hydrochar on soil pH. Malghani et al. (2015) demonstrated that the application of corn silage hydrochar significantly increased coarse soil pH after applying one year. Malghani et al. (2015) reported a significant increase in the pH of coarse soil following the application of corn silage hydrochar for one year. However, the authors noted that the pH rise may depend on the type of soil, as hydrochar did not result in a noticeable change in pH after one year of application to fine soil in the same study. Electrical conductivity (EC) is used as an important soil parameter for determining salinity levels (Khosravi et al., 2022). According to Yin et al. (2022), the addition of 1% hydrochar produced from cow manure and reed straw significantly increased soil EC from 152 s cm^{-1} to 788 s cm^{-1} and 1698 s cm^{-1}, respectively. However, a study by Chen et al. (2022) has demonstrated that, after the application of 2%, 4% and 6% hydrochar, the EC of a pH 9.83 soil decreased from 628 s cm^{-1} to 604, 581, and 568 s cm^{-1}. A possible explanation of differences in previous studies may vary depending on the materials for making hydrochar. The presence of ash on the surface of hydrochar is associated with its electrical conductivity, with hydrochar containing a high amount of ash displaying high electrical conductivity (Mohammadi et al., 2022). Cation exchange capacity (CEC) is an important indicator of soil quality for determining soil fertility (Sharma et al., 2015). As reported by Ebrahimi et al. (2022), with the addition of sludge hydrocahr to soil, soil CEC increased from 8.0 to 11.50 cmol kg^{-1}. In contrast, Sun et al. (2022) observed no significant change in soil CEC values following the application of 1% cow manure hydrochar, while 1% reed straw hydrochar decreased soil CEC from 14.8 to 4.75 cmol kg^{-1} in the same study. It is generally believed that the impact of hydrochar on soil CEC depends on hydrochar's feedstock and HTC manufacturing conditions. Compared to sludge, lignocellulose-derived hydrochar exhibited a higher CEC (Sun et al., 2020). Ebrahimi et al. (2022) also indicated that hydrochar produced at 180 °C has a greater CEC than hydrochar produced at 240 °C.

Hydrochar addition is considered to improve soil fertility. According to previous studies, hydrochar as a soil amendment increased soil's fertility in two ways. Firstly, hydrochar

contains several nutrients, including Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), and Magnesium (Mg), as well as some microelements, such as Chromium (Cr) and Nickel (Ni) (Melo et al., 2019). However, Novak et al. (2014) report that hydrochar has minimal impact to enhance soil fertility as a fertilizer. Secondly, hydrochar possessing a porous structure that can hold the nutrients in the soil, thereby reduce nutrient loss. Hydrochar can be a nutrient carrier that slowly release the nutrients into the soils to promote plant growth (Maghsoodi et al., 2020; Wu et al., 2021). In addition, the effect of hydrochar on soil nutrient content is influenced by the raw materials and the processing conditions (de Jager et al., 2020). Kumar et al. (2020) indicated that hydrochar produced from manure has higher nutrient contents compared to plant biomass-based hydrochar. According to Xu et al. (2022), the total phosphorus (P) of hydrochar increased with increasing HTC process temperature (180, 220, and 260 °C). In contrast, the total nitrogen (N) and total potassium (K) of hydrochar decreased as the temperature increased. This result was also confirmed by Xiong et al. (2021). Furthermore, as the HTC processing temperature increases, the porosity of hydrochar decreases (He et al., 2016), thereby further restricts the ability to absorb and release nutrients.

2.2.3 Effect of hydrochar on soil biology properties

Microorganisms in soil play a crucial role in nutrient cycling and soil quality, and further impact on plant growth and production yield (Dai et al., 2021; Prasad et al., 2021). Several studies to date have investigated the effect of hydrochar on soil biology characteristics. In a study conducted by Rillig et al. (2010), the authors found that with application 20% hydrochar into soil, the arbuscular mycorrhizal (AM) root colonization was significantly increased. Conversely, George et al. (2012) reported hydrochar derived from spent brewer's grains had negative effect on AM-fungal root colonization. The authors did not provide a clear explanation for the contrast observation between the two studies. However, it is noteworthy that the feedstock used in the two studies differed. Specifically, one study used beet root chips, while the other used spent brewer's grains. Similarly, for microbial community composition, the addition of hydrochar produced from corn silage detected reduced the abundance of *Acidobacteria* by 15.8%

and *Firmicutes* by 4.3%, while the abundance of *Bacteroidetes* was increased by 11% and *Proteobacteria* was increase by 19.9% compared to control (Andert and Mumme, 2015). Sun et al. (2020) reported that the application of hydrochar at a rate of 1.5% had a negative impact on fungal richness and diversity, while it increased bacterial richness and diversity. This phenomenon could be attributed to the pH of hydrochar, as the abundance and diversity of bacteria in soil tend to increase as soil pH increases, whereas the abundance and diversity of fungi tend to increase as soil pH decreases. A study carried out by Hu et al. (2014) also confirmed this finding, the abundance and richness of bacterial increased with addition of alkaline biochar.

2.3 Effect of application of hydrochar on plant growth

Apart from the impact of hydrochar on soil properties, the effect of hydrochar on crop growth has also been widely studied. The following section focuses the impact of application of hydrochar in growing media on plant germination, growth, and yield.

In a recent study, Islam et al. (2021) observed that chicken feather hydrochar had an inhibitory effect on seeds germination of earleaf acacia (*Acacia auriculiformis*). This study explained that with application of 0.125, 0.25, 0.50, 1 and 1.5% (w/w) feather hydrochar, the germination index (GI) decreased with increasing application rate and no seed germination was observed at the 1% and 1.5% application rate. Based on literature, few studies have found that hydrochar has positive effect on seeds germination. For example, Belda et al. (2016) found that seeds germination rate of myrtle (*Myrtus communis* L.) and mastic (*Pistacia lentiscus* L.) increased 13% and 18% when applied with 10% (v/v) forest waste based hydrochar. The authors also observed that the seeds germination was negatively impacted at higher application rate (25% and 50%). In contrast, de Jager and Giani (2021) used hydrochar derived from biogas digestate for soil improvement, they found that there was no significant difference on seeds germination rate in Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*) in different application rates (5%, 10%, 20%, and 30%) compared to control soil. Hydrochar addition that has inhibitory effect on plants, has been demonstrated in previous studies, including lettuce

(*Lactuca sativa* var. *longifolia*) (Cervera-Mata et al., 2021), French marigold (*Tagetes patula*) (Roehrdanz et al., 2019) and tomato (*Solanum lycopersicum* L.) (Fornes et al., 2017). Additionally, several studies have found that adding hydrochar to soil can promote plant growth. The yield of soybean (*Glycine max* (L.) Merr.) increased 29%-40% with the application of hydrochar (Egamberdieva et al., 2020). As has been previously shown, hydrochar was considered to have negative effect on seeds germination and plant growth in most of cases. The effect of hydrochar as soil amendments on plant growth in recent studies are summarized in Table 2.1.

Table 2.1. The effect of hydrochar as soil amendments on plant growth.

Hydrochar feedstock	Application rate	Plant	Plant response	References
Municipal green waste	5, 10, 15 and 20% (w/w)	lettuce	Seeds germination decreased with increasing hydrochar concentration	(Puccini et al., 2018)
Hickory wood, bagasse, and bamboo	0.2 g	Brown top millet (*Urochloa ramosa*)	Germination of seeds was inhibited, but not statistically significant No negative effect on plant growth	(Sun et al., 2014)
Orange peel	0, 5, 10, 25% (diluted) 5% (w/w)	Rocket (*Eruca sativa*), lettuce, maize (*Zea mays* L.)	Decreased seeds germination, Slightly decreased maize yield	(Kalderis et al., 2019)
Freshwater sludge	2 mL leachate	Wheat (*T. aestivum*)	Increased seeds germination index	(Zhang et al., 2021)
Sugarcane bagasse, hickory, and peanut hull	0.2 g	Brown top millet (*Urochloa ramosa*)	No statistically difference on seeds germination	(Fang et al., 2015)
Sugar beet,	10 g	Corn (*Zea*	Inhibited seeds germination	(Maletić et

Sliver grass		*mays*)		al., 2022)
Forest waste	0, 10, 25 and 50%	Pot marigold (*Calendula officinalis*), Garden Petunia (*Petunia hybrid*)	Germination and plant growth were increased at the dose of 10% and 25%, decreased at the dose of 50%	(Fornes and Belda, 2018)
Date palm leaflets	0.2 g	lettuce	Inhibited seeds germination and plant growth	(Wabel et al., 2019)
Chinese cabbage residue	1:100, w./v.	cabbage (*Brassica bara* L.)	Increased plant growth	(Wang et al., 2022)
Maize silage	30t ha^{-1}	Poplar (*Populus alba* L.)	Increased plant biomass production	(Baronti et al., 2017)
Spent brewer's grains	0, 5% and 10% (w/w)	Alfalfa (*Medicago sativa*)	No significant effect on root biomass, root length Decreased shoot dry biomass	(George et al., 2012)
Sawdust	0, 5 and 15% (w/w)	Rice (*Oryza sativa*)	Increased plant yield	(Hou et al., 2020)
Sewage sludge	0, 0.8 and 4% (w/w)	Perennial ryegrass (*lolium perenne*)	No significant effect on seeds germination Increased plant biomass at lower production temperature	(Paneque et al., 2019)
Biosolids and urban waste	50% (v/v)	Perennial ryegrass (*lolium perenne*)	Increased biomass production	(Álvarez et al., 2017)
Sugar beet pulp and	10 Mg ha^{-1}	Sugar beet (*Beta*	Reduced initial sugar beet growth	(Gajić and Koch,

beer draff		*vulgaris* L.)		2012)
Cow manure and reed straw	1% (w/w)	Lettuce	Cow manure hydrochar addition increased fresh biomass and plant height Reed straw hydrochar decreased fresh biomass	(Yin et al., 2022)
Sewage sludge and manure	2.0, 5.9, 9.8, 19.6, and 29.4 t ha^{-1}	Lettuce	Increased plant biomass production	(Huezo and Shah, 2022)

2.4 Phytotoxicity of hydrochar

The inhibitory effect of hydrochar on plants growth can be attributed to the phytotoxic compounds formed during hydrothermal carbonation (Bargmann et al., 2013). So far, it is not yet clear whether the composition of phytotoxicity is a single harmful substance or an accumulation of different harmful substances (Hitzl et al., 2018a). However, in previous studies, some harmful substances such as heavy metals, dioxins or polyaromatic hydrocarbons (PAH) were detected. Besides, phenolics (e.g. guaiacol or 3-methoxyphenol) are toxic substances that have been identified in water phase substances derived from the HTC process (Fornes and Belda, 2018). The difficulty of identifying harmful substances in hydrochar is due to many chemical reactions in the HTC process that have not yet been determined. Also, there is a large variety of raw materials that have been used to produce hydrochar. The characteristics of the different carbon materials from the different raw materials vary significantly, which has also caused difficulties in the identification of harmful materials (Ren et al., 2017). The analysis of hazardous substances can be made more difficult by the different production processes of hydrochar at a various range of temperatures and pressures (Zheng et al., 2019). It has been reported that the phytotoxicity of hydrochar decreased with the increasing HTC temperature. Lang et al. (2019) conducted an experiment to investigate the effect of HTC temperature on PAHs, they observed that with an increase in temperature from 180° to 220 °C, the total

PAHs content in the hydrochar decreased from 8182.21 to 3327.65 μg/kg. The result could be attributed to the lower boiling temperature of PAHs, leading them to transform into gas as the temperature of the HTC process increases.

2.5 Approaches for hydrochar phytotoxicity reduction

Given the discussion in the previous section, hydrochar has been identified as a controversial product in the context of agricultural applications. On the one hand, hydrochar has positive effects on soil properties. In some studies, it also exhibited a growth-promoting effect on plants. On the other hand, hydrochars exhibited phytotoxic potential in most studies, which has cast doubt on the certainty of using hydrochar in agriculture. Therefore, many studies have been conducted to remove phytotoxic compounds retained on the hydrochar, making it suitable for plant growth. At present, hydrochar phytotoxicity reduction approaches can be classified into three: (1) physical treatment, (2) chemical treatment, and (3) biological aging treatment.

2.51 Physical treatment

Breulmann et al. (2018) carried out a study to investigate the effect of pre-treatment with deionized (DI) water on hydrochar phytotoxicity and observed that water-washing efficiently reduced the inhibitory effect on the germination of cress (*Lepidium sativum* L.). The germination rate of hydrochar without pre-treatment was at 80%, while water-washed (60 mins) hydrochar increased the germination rate to 96%. Islam et al. (2021) conducted a similar germiantion experiment compared to fresh hydrochar, distilled water-washed hydrochar had a relatively lower inhibitory effect on seed germiation. However, water-washed hydrochar still inhibited seeds germination compared to control and water washing might not be sufficient to remove all phytotoxic compounds. The authors suggested that the fresh weight of seedlings increased but not significantly cpmpared to control. The explanation of water wash based removal of the phytotoxity was posssibly attibuted to the fact that most phytotoxic compounds were thought to be water-soluble (Bargmann et al., 2013). It is noted that the nutrient contents of hydrochar could be also lost during water washing treatment (Wabel et al., 2019).

Guan et al. (2021) discovered that the surface area of hydrochar increased after 90 freeze-thaw cycles (-20°-25 °C). In the same study, the authors also observed that the surface area of hydrochar increased when hydrochar was cycled 90 times at 70 °C (12 hours) and 25 °C (12 hours). There are no documented report that have investigated the effect of freeze-thaw and high temperature cycles on phytotoxicity reduction, but it has been reported that the increase in biochar surface area promoted the sorption of toxic compounds and thereby, reducing the phytotoxicity (Oleszczuk and Kołtowski, 2018). Therefore, further research is needed to investigate the effect of freeze-thaw and high-temperature cycles on the phytotoxicity of hydrochar.

2.5.2 Chemical treatment

Fornes and Belda (2017) performed a study that revealed hydrochar derived from forest waste had a negative impact on pea (*Pisum sativum* cv. Progress) and tomato seedling growth. The authors used nitric acid to remove the negative effects of hydrochar, and they concluded that the acid treatment successfully reduced the phytotoxicity, and the nitric acid-treated hydrochar significantly improved seedling development. In another study by Fan et al. (2021), tetrahydrofuran was used for hydrochar washing, and they concluded that washed-hydrochar had no significant negative effect on wheat seed germination compared to fresh hydrochar. However, this study found a lower value of roots and shoots length in treated hydrochar. Additionally, it is worth mentioning that the wastewater generated from washing may cause potential environment concern and require further treatment.

Pyrolysis was used to improve hydrochar in the study by Bahcivanji et al. (2020), hydrochar samples were pyrolyzed at 350 °C and 550 °C for 1, 3, and 5 hours. The results suggested that all pyrolyzed hydrochars increased the germination index of zucchini (*Cucurbita pepo*), with the highest germination index observed in hydrochar pyrolyzed at 550 °C for 3 hours. In addition, it is interesting to note that treated hydrochar did not display a stimulating effect on plant growth. Additionally, Hitzl et al.

(2018) was also found that the phytotoxicity of hydrochar can be removed through thermal treatment at 275 °C for 1 hour.

2.5.3 Biological aging treatment

Compared to physical and chemical treatment, biological aging treatment is considered as an environmentally friendly, lower-cost, and energy-saving way to remove phytotoxic compounds in hydrochar, making it suitable in agricultural application (Yu et al., 2019). Hydrochar was kept at a natural environment to undergo natural aging, which is the simplest form of biological aging. Puccini et al. (2018) performed a research based on the effect of naturally aged hydrochar on seed germination and seedling growth of lettuce (L. *sativa* var. Capitata). The results revealed a reduced inhibition effect on seed germination and seedling growth. Also, compared to fresh hydrochar, naturally aged hydrochar increased seed germination rate and seedling's root length. According to Bargmann et al. (2013), volatile compounds were present in fresh hydrochar. Thus, the inhibitory compounds of naturally stored hydrochar could be released into the air.

Pre-incubation of hydrochar in soils has been found to reduce the phytotoxic effect. A study performed by Busch et al. (2012) found that the inhibitory effect of hydrochar on barley (*Hordeum vulgare*) growth was short-term and not persistent. At the second harvest, hydrochar was observed to significantly promote plant growth. Similar result was obtained in Schimmelpfennig et al. (2014) where the inhibitory effect on perennial ryegrass (L. *perenne*) was alleviated when hydrochar was mixed with slurry and incubated for 3 months. This phenomenon could be attributed to the degradation of phytotoxic components by microorganisms in the soil. Additionally, microbial aging methods with biogas slurry was applied to investigate the effect on plant growth in Hou et al. (2020), the results showed that the yield of rice (*Oryza sativa* L.) was increased by treated with microbial aged hydrochar compared to control. But in same study, no inhibitory effect on plant growth was observed under growing media treated with fresh hydrochar. A study conducted by Roehrdanz et al. (2019) was also inspired by microbial aging method. In their study, hydrochar was mixed with compost in 50:50 ratio as

growing medium for French marigold growth. The results showed that co-composted hydrochar significantly improved the plant growth and increased the dry weight of plant.

2.6 Conclusion

Converting biomass waste to hydrochar via HTC offers a new option for resource recovery and addressing environmental concerns caused by irresponsible waste disposal. In this chapter, reviewing previous studies find that the use of hydrochar as soil amendment in practical application is a controversial topic. Hydrochar has encouraging prospects in terms of increasing the water retention and nutrient storage of soil. However, the phytotoxicity of hydrochar that negatively impacts plant growth has been confirmed in many studies. Fortunately, many researchers have found various ways to reduce or eliminate the phytotoxicity of hydrochar. Compared with freshly made hydrochar, modified hydrochar has received positive feedback on seed germination and plant growth. Each approach to reducing hydrochar phytotoxicity has its own advantages and disadvantages. Thus, future studies should focus on assessing the cost-effectiveness of these approaches and whether the process might bring in secondary pollution. Based on the current literature review, microbial aging is considered as the most promising method for improving the performance of hydrochar as a soil amendment. There are also a few studies that focus on simultaneously improving the physiochemical properties of hydrochar and removing its phytotoxicity. However, there is a lack of research comparing the effects of different modification methods on the physiochemical properties of hydrochar as well as the impacts of different modified hydrochars on plant growth. Thus, research is needed to address these challenges.

Chapter 3 Kale Seed Germination and Seedling Growth are affected by different Methods used to Age Hydrochar

3.1 Abstract

Hydrochar derived from hydrothermal carbonization (HTC) has been recognized as a potential absorbent and horticultural substrate. However, its practical application has been limited due to its low adsorption capacity and negative effects on plant growth. To address these issues, three pre-treatment methods (water washing, microbial aging, and freezing-thawing aging) were employed to further improve the physical structure and chemical properties of hydrochar. A seed germination test with kale (*Brassica oleracea* var. *acephala* DC.) was conducted to evaluate the phytotoxicity of modified hydrochars. The results showed that the pre-treatment, especially microbial aging, influenced the physicochemical properties of the hydrochar. Specifically, under microbial aging, the bulk density of microbial-aged hydrochar (MHC) decreased by 8.1%, the porosity increased by 24.8%, and the water-holding capacity increased by 36.54% compared to fresh hydrochar (FHC). Moreover, the surfaces of MHC and freezing-thawing aged hydrochar (FTHC) were observed with rough and cracked surfaces and macro pore structures. Fourier transform infrared (FTIR) spectroscopy revealed that the functional groups intensities of the four hydrochar materials varied, and that MHC and FTHC had more oxygen-containing groups than the others. Additionally, the surface areas of MHC and FTHC increased by 318.64% and 238.98% compared to FHC, respectively. The seed germination test indicated strong inhibitory effect of FHC, while MHC significantly ($P<0.05$) improved the seed germination rate and root development. Therefore, it can be concluded that microbial aging has the potential to enhance the hydrochar physiochemical features, and to minimize its seed germination-inhibiting ability. Further studies should consider optimizing the aging process.

Keywords: waste treatment; hydrochar; hydrothermal carbonization; soil amendment

3.2 Introduction

Horticultural crops are essential for human life and well-being, providing vital nutrients and playing a crucial role in maintaining a healthy diet and preventing malnutrition (Jiang et al., 2022; Khan et al., 2020). Among them, kale (*Brassica oleracea* var. *acephala* DC.) is a widely cultivated and consumed horticultural product worldwide, and it is also known as a functional food. Kale can be grown and used in various forms, including as microgreens (which are edible seedlings), baby greens (young plants), or mature plants. The productivity and quality of kale can be influenced by a variety of factors, such as the quality of the growing medium and environmental stressors. For example, peat is considered a fundamental component of horticultural substrates, but its resources are limited (Roehrdanz et al., 2019). Currently, hydrochar has gained more attention as a potential soil amendment and horticultural substrate.

Hydrochar is an emerging solid material created from hydrothermal carbonization (HTC) that can be used for a broad range of applications, including agricultural soil amendment, carbon sequestration, and carbon absorption (Kambo and Dutta, 2015). As a thermochemical process, HTC is carried out under high temperature and pressure conditions to convert the biomass into a coal-like product (hydrochar), which is characterized by its carbon-rich content and porous structure (Fang et al., 2015).

Currently, the majority of research has been conducted and proven that biochar is a promising absorbent and soil amendment (Berslin et al., 2022; Ding et al., 2016; Monisha et al., 2022).. Compared to pyrolysis biochar, HTC has been shown to offer multiple advantages, such as allowing for the use of wet and dry biomass as feedstocks due to the presence of water as a reaction medium, which means the biomass does not need to be pre-dried (Libra et al., 2011). Meanwhile, as the HTC has lower emissions and requires less energy, it creates less pollution with a higher hydrochar yield (Zhang et al., 2019). However, hydrochar has poor sorption characteristics compared to biochar, such as small surface area and pore volume, which stem from its production process conditions (Fang et al., 2018). These characteristics also limit the utilization of hydrochar as an adsorbent, although previous studies have found that hydrochar possesses a range of sorption abilities and can be used as a low-cost adsorbent in certain areas (Fernandez et al., 2015;

Georgiou et al., 2021; He et al., 2020; Tran et al., 2020; Yu et al., 2019). For example, rare-earth ions can be efficiently removed from wastewater by hydrochar produced from kitchen waste (Yu et al., 2019).

Besides its absorption values, hydrochar is considered a potential soil amendment due to its nutrient-holding capacity and the ability to improve soil physicochemical properties such as water-holding capacity, water-stable aggregation, pH, and cation exchange capacity (de Jager and Giani, 2021; Khosravi et al., 2022). These characteristics of hydrochar mainly depend on porosity and surface chemical properties (Tasca et al., 2019). For instance, nutrients in soils can be adsorbed onto the surface pores of hydrochar and thereby, reducing nutrients lost from soil through leaching (Khosravi et al., 2022). Notwithstanding, the presence of toxic compounds in hydrochars, such as phenolic compounds and dioxins pose some potential risks for inhibiting plant growth and decreasing productivity (Bargmann et al., 2013). However, previous reports have shown that it is necessary to pre-treat hydrochar to reduce its phytotoxicity and render it suitable for use in agricultural soil (Hitzl et al., 2018). From the above, it can be concluded that hydrochar has the potential to serve as a soil conditioner, an adsorbent, or be used in other applications. However, there are still issues with its physicochemical properties that limit its performance. Generally, for good-performing hydrochar, additional treatment steps are required to modify the surface structure and chemical properties (Kambo and Dutta, 2015).

In previous studies, physical and chemical methods were used to modify various char products (Fornes and Belda, 2017; Hao et al., 2013; Hitzl et al., 2018; Román et al., 2013). For instance, the surface areas of hydrochar produced from waste biomass such as sunflower (*Helianthus annuus*) stem, walnut (*Juglans regia*) shells and olive (*Olea europaea*) stone were increased following activation in carbon dioxide, which consequently improved its absorbent ability (Hao et al., 2013). Also, Zhu et al. (2014) reported that the surface area of hydrochar can be modified through radiation to increase its absorption capacity. In the present study, we focused on aging method, with the aim of tailoring the surface structure and chemistry. This method offers several advantages

including lower costs, preventing or minimizing pollutant gas emissions, and limiting the generation of chemical waste. Hydrochar is known to age naturally when in contact with air, soil and microbes (Mia et al., 2017). Microbes were used to accelerate the biochar aging process during which oxygen-containing functional groups were increased as a result (Quan et al., 2020).

Moreover, biochar can be subjected to freeze-thaw aging to improve surface area and absorption capacity (Wang et al., 2021). There is a lack of studies on the aging of hydrochar to date, particularly with regard to improving its physicochemical properties and surface structure. The aging method is considered promising for revealing the full potential of hydrochar because of the energy-saving and eco-friendly benefits generated by the production of hydrochar and aging it. Therefore, this study first time proposes a comparison of the effects of water washing, microbial aging, and freezing-thawing aging on the physical and chemical properties of hydrochar, as well as their impact on seed germination. It is expected that microbial hydrochar (MHC) will exhibit the best performance, as the surface porosity and physicochemical properties of biochar were enhanced by microbial activities (Mia et al., 2017).

To obtain better properties of hydrochar and facilitate its application in horticultural production. This study aims to ascertain the effect of three pre-treatment methods (water washing, microbial aging and freezing-thawing aging) on the surface structure and the physicochemical properties of fresh hydrochar produced from coffee (*Coffea arabica*) grounds, as well as the effects of these aging methods on kale seed germination and root development.

3.3 Materials and Methods

3.3.1 Location and materials

The study was conducted in the Department of Engineering and the Department of Plant, Food, and Environmental Sciences. Hydrochar was prepared from coffee (*Coffea arabica*) grounds obtained from Tim Hortons in Truro, NS. There were four hydrochar

materials used in this study as follows: fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial-aged coffee grounds hydrochar (MHC) and freezing-thawing aged hydrochar (FTHC). Garden Club sheep manure compost was purchased from Canadian Tire Corporation, Truro, NS, Canada. Kale seeds were purchased from Halifax Seeds Company (Halifax, NS, Canada).

3.3.2 Hydrochar production

Hydrothermal carbonization experiment was carried out in a stainless-steel autoclave (Parr Instrument, 4590 micro-reactor (Moline, Illinois, USA)) fitted with an A2140HC stirring mechanism and a 4848-reactor controller. Coffee grounds (70 g) and 280 mL of distilled water were transferred to the reactor and then sealed. The sealed reactor was positioned into the autoclave support stand and purged using nitrogen gas, and then re-pressurized to 1 MPa. Inductive heating was applied to the reactor at 210°C for 1 hour while stirring. Once the reaction was completed, the reactor temperature was cooled down to room temperature (approximately 25°C) using a water bath. Following this the gas in the reaction vessel was vented. The reactor was opened following by filtration process to separate the hydrochar solid and the process water. The hydrochar was oven-dried at 105°C for 12 hours. Finally, the dried hydrochar and process water were collected in containers and stored in shaded areas for future use.

3.3.3 Aging of hydrochars

The hydrochar materials were aged according to the following procedure. To make WHC, fresh hydrochar material and distilled water were poured into a container with distilled water at a 1:10 hydrochar/distilled water ratio. The mixture was washed well and stirred continuously for 1 hr. The assumption was that washing with distilled water will leach out hydrophilic phytotoxic compounds from the hydrochar to improve its efficacy on plants (Bargmann et al., 2013; Yu et al., 2019). The WHC was obtained by filtration and air-dried. With respect to MHC, 1 L of distilled water was mixed with 200 g of sheep manure compost and stirred for 24 hours at 2000 rotations per minute using a DLM186X1 Isotemp stirring plate (Fisher Scientific Inc., Markham, ON, Canada). The

assumption was that manure compost like any other compost is rich in a beneficial microbiome that can be used to inoculate growing substrates to benefit plants as recently reported by Abbey et al. (2022). To inoculate FHC with microbial tea, dried 3 kg FHC was mixed with 2.4 L of microbial tea in a plastic container at 80% moisture content for 45 days in the shade area. To maintain moisture, the container was positioned away from light and covered with a thin film. Upon completion of the microbial-aged process, the MHC material was air dried and then stored in a sealed container. FTHC was prepared by mixing 3000 g fresh hydrochar with 2400 mL microbial tea at 80% moisture content. The mixture was then kept in the freezer (-15°C) for 5 hours followed by 19 hours in a 25°C room cycle for 45 days. After the inoculation, the FTHC was air dried and stored in a sealed container. The assumption was that the hydrochar particles disintegrate by the process of freezing and thawing leading to the creation of larger surface areas for nutrients adsorption and more active sites for microbiome activities (Cui et al., 2021; Junjie et al., 2021).

3.3.4 Hydrochar physical properties

3.3.4.1 Bulk density

The bulk density of the hydrochar samples were determined using a modified method as described by Kalderis et al. (2019). Briefly, hydrochar material was transferred and filled to a 50 mL laboratory tube followed by tapping the tube three times to compact the volume. The tube was then refilled to 50 mL by hydrochar material. The weight of the hydrochar material and tube was measured using an electronic MXX-412 Denver precision balance (Denver Instrument Company, CO, USA) with four replications. The bulk density of the hydrochar sample was determined using the following equation: Bulk density (g/mL) = $\frac{M_t - M_b}{V_t}$; where M_t is total mass of tube and hydrochar, M_b is mass of empty tube and V_t is the volume of tube (50 mL).

3.3.4.2 Porosity and water holding capacity

The total porosity and water-holding capacity were determined by the methods described in Lipiec et al. (2006). All determinations were performed quadruplicate. A 50-mL hydrochar samples was transferred to a plastic cell holder with two drainage holes at the bottom. The cell holder with the hydrochar was put on a plastic tray that was half-filled with distilled water to saturation for 6 hrs. The weight of saturated hydrochar and dry hydrochar were recorded and the total porosity was determined as follows:

Porosity (%) = $\frac{M_s - M_d}{V_t}$; where M_s is the weight of saturated hydrochar, M_d is the weight of dry hydrochar and V_t is the volume (50 mL).

The water holding capacity measurement was carried out using the gravitational water method. The saturated cell holder was drained naturally for 24 hrs under gravity and the weight of the drained hydrochar was recorded. The following formula was used to determine the water holding capacity.

Water holding capacity = $\frac{M_a - M_d}{M_d}$; where Ma is the weight of hydrochar material after drained, M_d is the weight of dry hydrochar material.

3.3.5 Surface morphology

Scanning electron microscope analysis (SEM) measurements were made to determine the changes of surface morphology and microstructure of hydrochar samples at the Department of Mechanical Engineering, Dalhousie University. Hydrochar samples were sputter coated with a 21-nm thickness layer of Au/Pd 80/20 to prevent charging during observation. The SEM analysis was then conducted with a scanning electron microscopy (SEM) (Zeiss Sigma 300 FESEM Oberkochen, Germany) at an acceleration voltage of 10 kV. The weight percent of surface elements of hydrochar samples were analyzed by an energy dispersive X-ray fluorescence spectroscopy (EDS) (Oxford Instruments, Britain). The surface areas of hydrochar samples were determined by using Gemini II – 2375 BET (Brunauer-Emmett-Teller) analyzer (Micromeritics Inc., Norcross, GA) at Department of Physics and Atmospheric Sciences, Dalhousie University. The method of Brunauer-Emmett-Teller (BET) was used to determine the surface areas with N_2 adsorption at 77 K. The determination was replicated three times.

3.3.6 Fourier transform infrared (FTIR)

The variation of functional groups after hydrochar samples were analyzed by fourier transform infrared (FTIR) at the spectral range of 4000 cm−1 to 500 cm−1. FTIR analysis was carried out in a PerkinElmer FTIR spectrometer (PerkinElmer, Inc., MA, USA) in the Department of Engineering, Dalhousie University Agriculture Campus. 5-10 mg of hydrochar samples were positioned on the diamond surface and pressed against a metal rod and all the spectra were recorded from 64 scans at 4 cm–1 resolution.

3.3.7 Hydrochars chemical properties

The pH, total dissolved solid (TDS), salinity and electric conductivity (EC) of hydrochar samples were determined with a multifunctional EC 500 ExStik pH meter (EXTECH Instrument, New Hampshire, USA). Briefly, 5-g hydrochar samples were mixed with 50 mL deionized water resulting in 1:10 mass/water ratio and thoroughly stirred for 1 hr using DLM1886X1 Isotemp stirring plate (Fisher Scientific Inc., Markham, ON, Canada). Finally, the hydrochar leachate was obtained by vacuum filtration for pH/TDS/salinity/EC measurement. The hydrochar leachate was also used for pouch seed germination test. All analyses were performed in triplicate.

3.3.8 Seed germination bioassay

One-way analysis was conducted in the Compost and Biostimulant Laboratory at Dalhousie University Agriculture Campus to investigate the effect of hydrochar leachates on kale seed germination and root growth using a modified method described by Islam et al. (2021). Fifteen kale seeds were placed evenly and carefully in a 16.5 × 18 cm CYG germination pouch (Mega International, USA), followed by treatment with 25 mL of hydrochar leachate. After that, the pouches were incubated in a dark at 22°C for 2 days after which they were transferred to a growth chamber with at an approximately 71% relative humidity, 25°/20°C day/night temperature regime, and 16/8 hrs day/night LED lighting. The pouch assay was designed in a completely randomized design (CRD) with three replications. Each pouch was added 15 mL of leachate to maintain adequate

moisture for seeds growth every two days. For the next seven days, the number of germinated seeds was recorded daily. Germination is generally considered to have occurred when the radicle emerges at approximately 2 mm in length. Germination rate (GR), germination index (GI), mean germination time (MGT), and germination energy (GE) were also determined. On day 9, the kale seedlings were gently removed from the pouch and placed in the plastic tray of STD4800 Epson Perfection V850 Pro Scanner (Regent Instruments, QC, Canada) and WinRHIZO™2000 software was used to detect the morphology of kale seedlings (root length, root surface area). Germination rate and other relative germination indices were calculated using the following equations (Yang et al., 2015; Yousefi et al., 2017).

Germination rate (GR) $= \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100$

Germination energy (GE) $= \frac{\text{number of seeds germinated}}{\text{number of total seeds per treatment after germination for 4 days}} \times 100$

Germination index (GI) = $\sum$ (Gt/Tt); where Gt is the germinated numbers on day t and Tt is the number of days.

Seed vigour index (SVI) = Germination rate × seedlings length

3.3.9 Statistical analysis

Analysis of variance was performed to determine differences in the treatments at a significant different level of 5%. Tukey's test was used to compare and separate the treatment means when the ANOVA suggested $P \leq 0.05$. All statistical analyses were performed using Minitab software version 20 (Minitab Inc., State College, Pennsylvania, USA). All graphs were drawn by Origin Pro 8.50 (Northampton, Massachusetts, USA).

3.4 Results

3.4.1 Effect of different pre-treatment methods on physical properties of hydrochar

The effects of aging on hydrochar physical properties are shown in Fig 3.1. The difference in letter indicates that the treatments differ significantly ($P<0.05$). Among all modified hydrochars, MHC showed the most significant ($P<0.05$) changes in physical properties. Compared to the control, MHC exhibited an 8.1% decrease in bulk density (0.37 to 0.34 g cm-3), a 24.8% increase in porosity (73.36% to 91.64%), and a 36.4% increase in water-holding capacity (148.96% to 203.39%). The porosity and water-holding capacity of FTHC also increased significantly ($P<0.05$), but were slightly inferior to MHC, with increases of 11.3% and 18.6%, respectively, compared to FHC. Moreover, WHC showed a significant ($P<0.05$) decrease in bulk density, which decreased from 0.37 g cm-3 to 0.35 g cm-3, representing a 5.4% decrease. The porosity and water-holding capacity of WHC also increased, by 2.32% and 6.93%, respectively, but this was not statistically significant ($P > 0.05$).

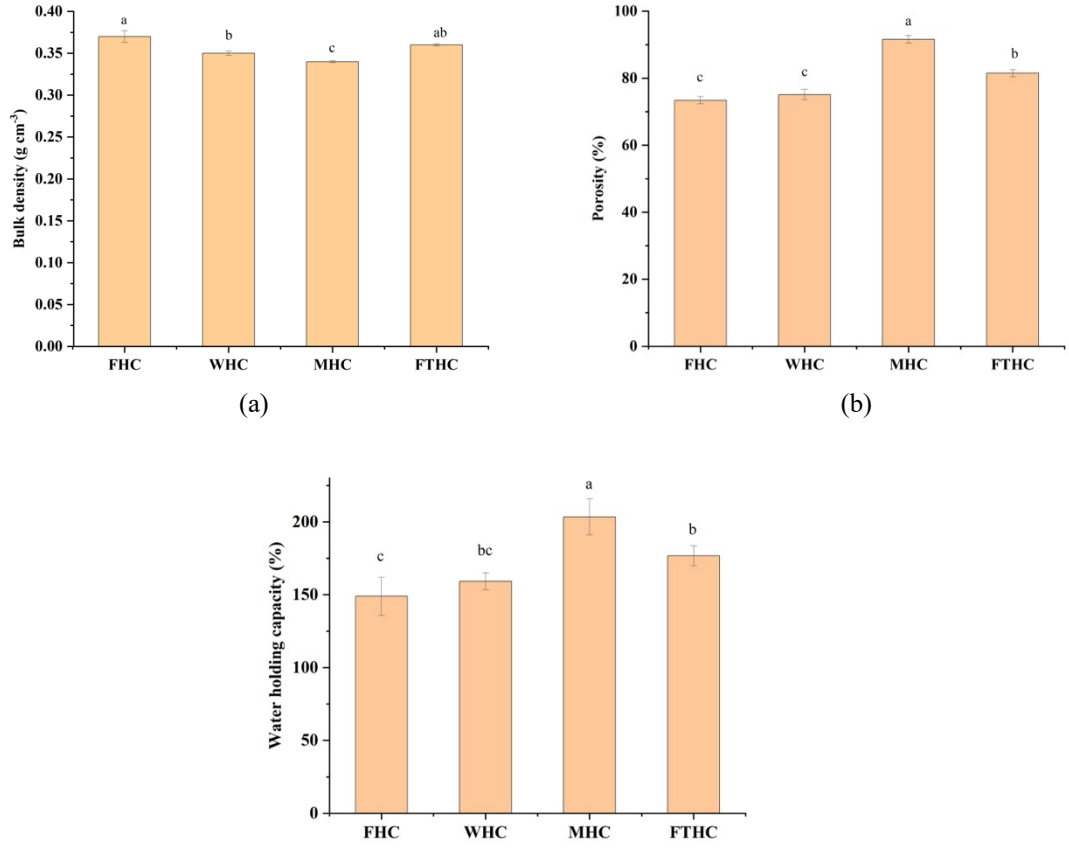


Fig. 3.1. Physical properties of hydrochar subjected to different aging processes. Values represent mean of four replicate analyses. Error bars represent as standard deviation.

Statistical difference between treatments is represented by lower-case letters. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC).

3.4.2 Effect of different pre-treatment methods on morphological characteristics of hydrochars

As shown in Fig. 3.2, the SEM images revealed that the morphology of hydrochar aged in various ways changed to varying degrees. The surface morphology of WHC and FHC appeared identical. On the surfaces of FHC and WHC, we can clearly observe compact structures and smooth surfaces with small-diameter pores. In contrast, the surface morphologies of MHC and FTHC were dramatically altered (Fig. 3.2). The surfaces of MHC and FTHC were rough and exhibited larger regular network structures compared to FHC and WHC. In addition, it was evident that MHC and FTHC had more pores with well-developed structures than FHC.

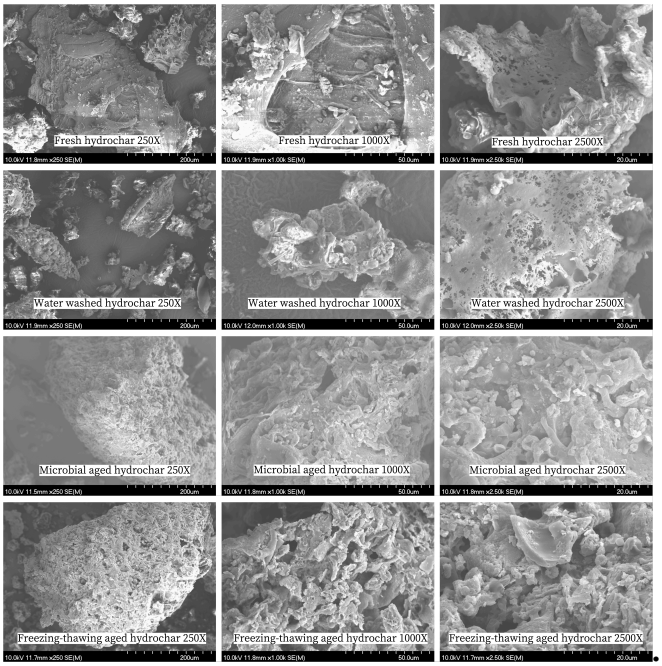


Fig. 3.2. Scanning electron microscope (SEM) images of fresh hydrochar, water washed hydrochar, microbial aged hydrochar and freezing-thawing aged hydrochar with magnifications of 250X, 1000X and 2500X.

By employing X-ray fluorescence spectroscopy (EDS) investigation, the elemental surface composition and dispersion of hydrochars were determined (Table 3.1). The EDS

examination revealed that the primary elements were carbon (C), nitrogen (N), and oxygen (O). The C, N, and O percentages in FHC were 76.8%, 5.9%, and 17.3%, respectively. Compared to those of FHC, the weight of WHC elements was marginally altered. Compared to FHC, N and O in MHC increased from 5.9% to 8.4% and 17.3% to 22.2%; and for FTHC, N and O content increased to 6.9% and 21.1%, respectively. Furthermore, microbial and freezing-thawing aging resulted in a decrease in the C content, from approximately 77% to 69% for MHC and from 77% to 72% for FTHC (Table 3.1).

Table 3.1. Hydrochar carbon (C), nitrogen (N) and oxygen (O) contents as affected by different aging techniques.

Element	**FHC**	**WHC**	**WHC**	**FTHC**
C	76.8	75.7	69.3	72.1
N	5.9	5.9	8.4	6.9
O	17.3	18.4	22.4	21.1

Note: Carbon (C), Nitrogen (N), Oxygen (O), Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC).

Based on the BET analysis, the hydrochars experienced a remarkable change in surface area after aging (Fig. 3.3). Overall, the trend of BET surface area of hydrochars were MHC > FTHC > WHC = FHC. The BET surface area of FHC was 0.59 m^2 g^{-1}. As expected, the BET surface area of MHC and FTHC were increased by 3.2-fold and 2.4-fold, respectively, compared to FHC, with the values of 2.46 m^2 g^{-1} and 1.99 m^2 g^{-1}.

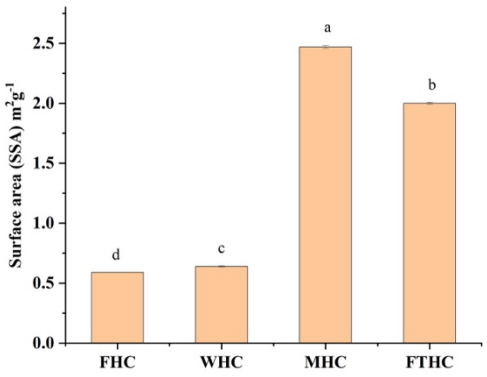


Fig. 3.3. BET analysis of the hydrochar derived from coffee grounds with different modification methods. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC). Error bars represent as standard deviation. Statistical difference between treatments is represented by lower-case letters.

3.4.3 FTIR analysis of hydrochars

The surface functional groups of the hydrochars were identified by FTIR as expressed in Fig. 3.4. Overall, there was no clear change in the major absorption peaks of the four hydrochar samples, but their intensities were different. The peak of 3345 cm^{-1} was attributed to the O-H stretching vibration (Nguyen et al., 2021). The peaks at 2928 cm^{-1} and 2870 cm^{-1} were attributed to aliphatic C-H stretching vibration peaks (Reza et al., 2015). The peaks at 1700-1630 cm^{-1} and 1457cm^{-1} were attributed to increased C=O (carbonyl, Quinone, ester, or carboxyl) functions and C=C vibrations, respectively (Wang et al., 2021). The peak at 1039 cm^{-1} was ascribed to C-O stretching vibration (Nguyen et al., 2021). The peak at 1171 cm^{-1} was attributed to the C-C stretching vibration (Nakkeeran et al., 2016).

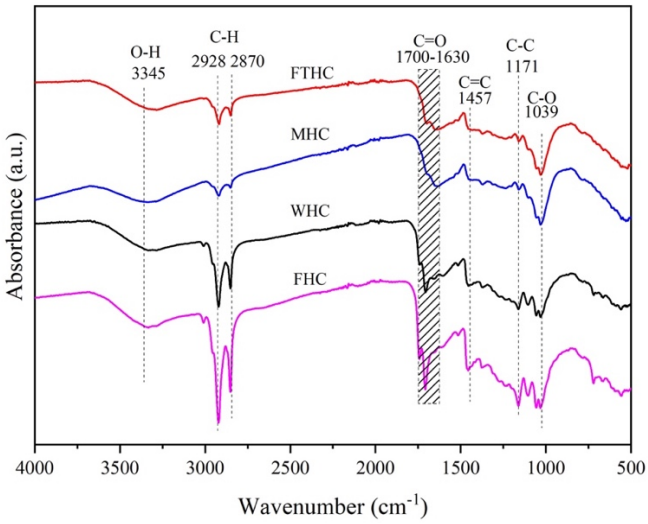


Fig. 3.4. FTIR spectrum of hydrochars subjected to different aging processes. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC).

3.4.4 Effect of pre-treatment methods on the chemical properties of hydrochar

The chemical properties of the hydrochars were significantly affected ($P<0.05$) by the different aging methods, as shown in Fig. 3.5a-d. As seen in Fig. 5a, the pH of all the modified hydrochars increased to varying extents, ranging between 4.1 and 5.8, with the highest increase observed in MHC, followed by FTHC and then WHC. The pH of MHC increased by approximately 42%, reaching 5.8, compared to FHC. Similarly, there was a noticeable increase in pH of FTHC, which was approximately 39% higher, at 5.7, than that of FHC. Comparatively, a clear pattern was observed for EC, salinity, and TDS of the hydrochars (Fig. 3.5b-d). WHC had the lowest TDS, at 126.9, compared to FHC, WHC, MHC, and FTHC. Additionally, MHC significantly ($P<0.05$) reduced EC, salinity, and TDS by approximately 33%, 35%, and 33%, respectively, compared to FHC. FTHC showed the smallest reduction in EC, salinity, and TDS, at 27%, 22%, and 21%, respectively, compared to FHC.

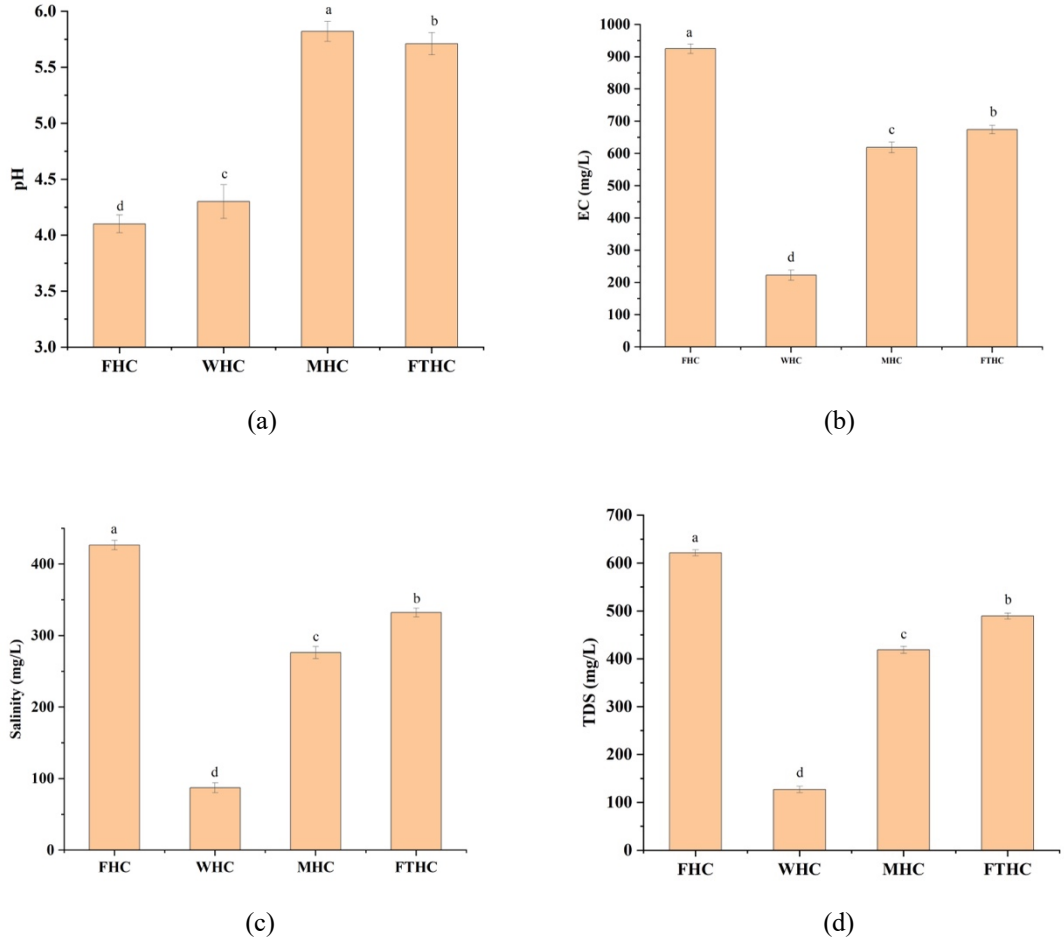


Fig. 3.5. Chemical properties of hydrochar under different modification methods: (a) pH, (b) electrical conductivity (EC), (c) salinity, (d) total dissolved solids (TDS). Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC). Values represent mean of three replicate analyses. Error bars represent as standard deviation. Statistical difference between treatments is represented by lower-case letters.

3.4.5 Effects of aged hydrochars on seed germination indices

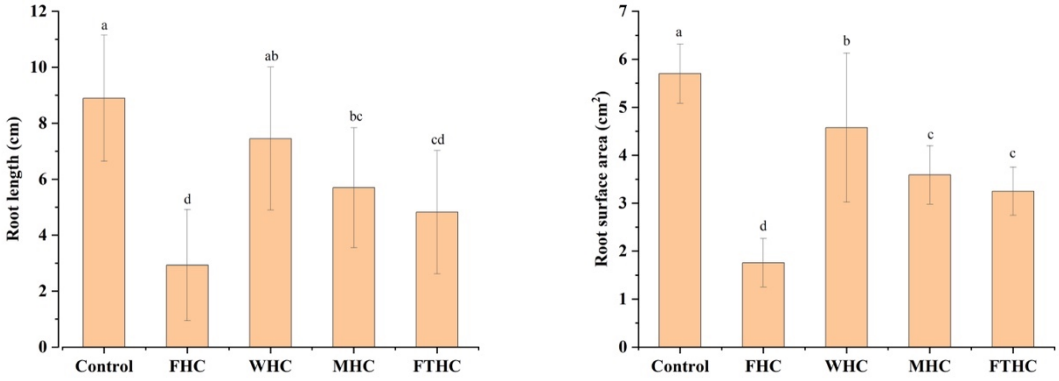
The overall trend for seed germination rate was MHC > control > FTHC > WHC > FHC (Table 3.2). FHC had the least average germination rate that significantly ($P<0.05$) different from the other aged hydrochars. In addition, germination energy (GE), germination index (GI) and seed vigour index (SVI) were used to determine seedling growth response. As shown in Table 2, the control treatment had the highest GE, GI, and SVI, respectively.

Table 3.2. The average germination rate (%), germination energy (%), germination index and seed vigour index of kale (Brassica oleracea var. sabellica) treated with different hydrochar leachates.

Treatment	Germination rate (%)	Germination energy (%)	Germination index	Seed Vigour Index
Control	82.2±10.18 a	64.4±0.20 a	13.49±3.16 a	9.02±2.76 a
FHC	28.9±19.20 b	6.7±0.07 b	2.52±1.54b	1.49±1.10 b
WHC	68.9±10.18 a	40.0±0.12ab	9.17±1.36 a	6.90±1.83 a
MHC	86.7±17.60 a	48.9±0.14 ab	11.81±2.77 a	7.13±2.40 a
FTHC	71.1±10.18 a	46.7±0.24 ab	10.17±2.33 a	5.31±1.07 ab
P-value	0.004	0.018	0.002	0.009

Statistical difference between treatments is represented by lower-case letters. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC).

Effects of hydrochar treatments on kale seed root development, as shown in Figs. 3.6 and Fig. 3.7. Control showed the best root development, while a significant ($P<0.05$) inhibition of root development was observed for FHC. Moreover, the three pre-treatment methods significantly reduced the inhibitory effect on root development compared to FHC. Among them, WHC had the smallest inhibitory effect, with a root length and root surface area of 5.7 cm and 3.6 cm2, respectively.



(a) (b)

Fig. 3.6. (a) Root length and (b) surface area of kale (Brassica oleracea var. sabellica) seedlings treated with different hydrochar leachates. Values represent mean of seven replicate analyses. Error bars represent as standard deviation. Statistical difference between treatments is represented by lower-case letters. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC). Error bars represent as standard deviation. Statistical difference between treatments is represented by lower-case letters.

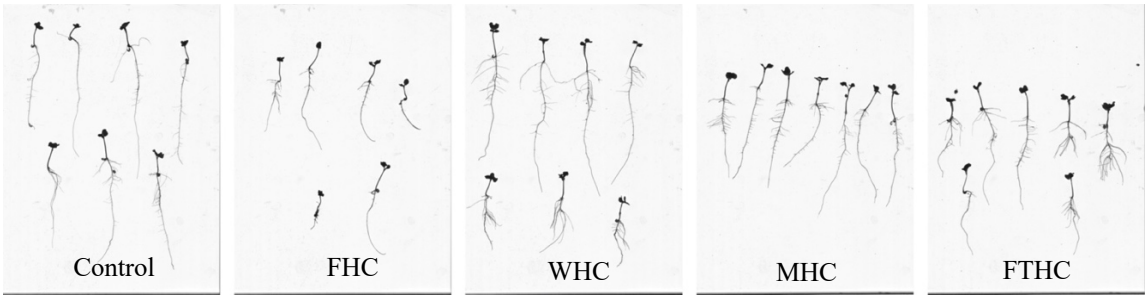


Fig. 3.7. Photo comparison of seedlings showing differences in size of kale (Brassica oleracea var. sabellica), as affected by different hydrochar leachates. Fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial aged hydrochar (MHC), and freezing-thawing aged hydrochar (FTHC).

3.5 Discussion

3.5.1 Explanation for physical properties changes

Water-holding capacity is an important soil indicator that affects soil health and soil quality (Michael, 2019). Due to the porous structure of hydrochar, people have been exploring the use of hydrochar for improving soil physical properties (Abel et al., 2013). Meanwhile, researchers have conducted a series of studies to optimize hydrochar and improve its pore structure (Chatir et al., 2022; Zhang et al., 2019). In this study, MHC

displayed the greatest changes in physical properties and was consistent with previous hydrochar modification studies (Hao et al., 2013; Zhu et al., 2014). This may be attributed to the structural changes in hydrochar caused by microbial degradation as the surface area increased (Hua et al., 2020). For FTHC, changes in physical properties can be associated with the freezing-thawing aging process, as according Liu et al. (2018), after 20 times of freezing and thawing cycles, the porosity of biochar increased by 3.2%, which resulted in improved water-holding capacity. However, it is important to note that the FTHC aging process also involves microbial activities (microbial tea was added); surprisingly, the physical properties of FTHC after freezing-thawing and microbial activities were slightly inferior to those of MHC (Fig.1). It is speculated that the improvement of the physical properties of FTHC was mainly due to the freezing-thawing process because the low temperature of FTHC during the aging period inhibits microbial activity (Panikov et al., 2006). There have been few studies conducted on the effects of washing on the physical properties of hydrochar. We speculate that the decrease in bulk density in the WHC could be attributed to washing off the ash on the surface of hydrochar. Overall, our results suggest that modified hydrochars, particularly MHC, are more effective for improving soil water retention and overall soil health.

3.5.2 Explanation Explanation for surface characteristics changes of hydrochar

In this study, the decrease in C content in MHC could be attributable to the effects of microbial digestion (Wang et al., 2021). In addition, earlier research conducted by Jing et al. (2021) demonstrated a reduction in C content of hydrochar after freezing and thawing. As a result of carbon loss, the O and ash contents of the samples increased relatively after aging, which is consistent with the results of this experiment. The increase in the O content in hydrochar means an increase in the hydrophilic oxygen functional groups, which subsequently enhances the hydrophilicity of hydrochar (Liu et al., 2019). The increased N content of MHC could be related to the effect of microbial activity (McMillan et al., 2007). Also, the increased N content of FTHC could be attributed to the freezing-thawing process. In a recent study by Zhou et al. (2011), they observed that the freezing-thawing process increased the amount of inorganic N in soils by 1.67–26.77

times. Additionally, Dalias et al. (2002) suggested that microbial activity and soil N cycling were inhibited at lower temperatures. Therefore, we hypothesized that freeze-thaw cycles will alter the physiochemical properties and microbial activities of the hydrochar. According to a prior study carried out by Sun et al. (2014), hydrochar could be regarded as an effective adsorbent since it contains more O and less C. The present study, therefore, provides additional evidence that MHC and FTHC have a superior adsorption capability.

A recent study showed that after microbial aging for 60 days, the surface area of the hydrochar increased by 2.2-fold compared to fresh hydrochar (Hua et al., 2020). This is consistent with the results of our study. The increase in microbial aged hydrochar BET surface area was associated with the release of dissolved organic matter on the surface of the hydrochar. During the microbial aging process, hydrochar loses an extensive amount of decomposable and soluble organic and inorganic components, which leads to an increase in surface area (Hua et al., 2020; Zhu et al., 2019). The BET surface area of FTHC was 1.99% m^2 g^{-1}, with a 2.4-fold increase over FHC. As pointed out by Dong et al. (2017), the infiltration of water leads to swelling followed by shrinking of pores of hydrochar due to temperature changes, thus breaking the surface of the hydrochar and increasing the surface area further. It is worth noting that an excessive number of freezing-thawing cycles can also damage biochar particles, resulting in a reduction in particle size due to the expansion of micropores (Liu et al., 2018). The increase in surface area of MHC and FTHC was also confirmed in the SEM analysis (Fig. 3.2), and the rough surface of MHC and FTHC helps to enhance the surface area. It was also observed that the BET surface area of WHC increased by approximately 7.6%. It can be speculated that the ash produced in the HTC process was washed off the WHC aged hydrochar leading to an increase in its surface area. According to Sun et al. (2014), surface area plays an important role in determining adsorption capacity of hydrochars, therefore, it can be concluded that MHC and FTHC have better adsorption capacity.

We observed that there was a considerable difference in intensity of the peaks at 2920, 2870 and 1171cm^{-1}. FHC exhibited the highest intensity, which indicated fresh

hydrochar exhibited higher abundance of C-H/C-C. The weak intensity of the other three hydrochar samples could be attributed to carbon (C) removal (Zhu et al., 2014). Compared with FHC, the peak at 1039 cm^{-1} illustrated that those aging methods could proliferate O-containing functional groups (C-O). At peaks of 1700-1630 cm^{-1}, the peaks became wider and shifted to the right (1648 cm^{-1}), which indicated that aging process may lead to the breakage of the C=O functional groups. The shift of peak at 1700 cm^{-1} can be ascribed to deprotonation of acid functional groups such as carboxylic acid to carboxylate per the increase in the pH of MHC and FTHC. Besides, the increased microbial abundance and activities might have further improved the contents of amide bonds leading to the observed differences in addition to the observed increase in N contents of the MHC and FTHC treatments.

3.5.3 Seeds Germination Indices

Bargmann et al. (2013) reported that hydrochar inhibited cress (*Lepidium sativum*) germination, which is consistent with our experimental results. Their study identified organic acids and phenols as phytotoxic compounds in hydrochar, an area that requires further attention in future research. Chemical analysis of hydrochar showed that FHC had a lower pH and the highest EC, which could disrupt the balance between soil water and soil nutrients, affecting plant growth (Hu et al., 2022). Bargmann et al. (2013) also found that water-washing hydrochar greatly reduced the negative impact on germination, as the phytotoxic compounds in hydrochar are mostly water-soluble. In our experiment, WHC had a considerable improved germination rate and root development compared to FHC, but still showed an inhibitory effect compared to the control. Therefore, future research should investigate different washing ratios and washing times.

MHC and FTHC showed some alleviation of inhibition on seed germination, which may be related to the effect of aging on the chemical properties of hydrochar. According to Msimbira and Smith (Msimbira and Smith, 2020), the optimal pH range for plant growth is between 5.5 and 6.5, as most mineral nutrients are available within this pH range. The rise in MHC pH could be due to the decomposition of acidic organic molecules by

microbes during the aging process, as previously reported by Yu et al. (2019). Furthermore, Roehrdanz et al. (2019) mentioned that microbial degradation may reduce harmful substances. Specifically, during microbial aging, the dissolved organic carbon (DOC), inorganic components, and some tar residues in the surface of hydrochar carbon skeleton are degraded, which leads to an increase in the surface area and porosity of the hydrochar. This, in turn, enhances its adsorption capacity, allowing for the adsorption of phytotoxic compounds and reducing their inhibitory effect. On the other hand, the increase in surface area and porosity provides more space for microorganisms to degrade harmful components in the hydrochar, ultimately resulting in a reduction in hydrochar's phytotoxicity and its inhibitory effect on plant germination and growth (Hua et al., 2020; Oleszczuk and Kołtowski, 2018). Our study also found that MHC had the highest germination rate, but its root development was not particularly good compared to the control and WHC. This could be attributed that seed germination and seedling growth are two distinct physiological stages (Fornes and Belda, 2017). Additionally, FTHC showed a noticeable increase in pH of approximately 39% compared to the control, contradicting the findings reported by (Dong et al., 2017; Zhu et al., 2019). This increase in pH may be related to the addition of micro-tea. MHC and FTHC were more suitable as soil amendments than FHC and WHC due to their low acidity levels. Further research is needed on the effect of microbial or freezing-thawing aging on salinity, EC, and TDS of hydrochar. There is currently no report on the effect of freezing-thawing aging on seed germination, and we can speculate that the reduced inhibitory effect of FTHC may be related to the microbial activities in the micro tea. Overall, the seed germination experiment confirmed the inhibitory effect of FHC on kale seed germination, and both washing and aging alleviated this inhibition to varying degrees.

3.6 Conclusion and Recommendation

Hydrochar exhibits considerable promise as a soil amendment and absorbent in agricultural and remediation applications. In this study, we conducted experiments to investigate the impact of three pre-treatment methods on hydrochar, focusing on changes in its physical and chemical properties, as well as its effects on kale seed germination.

Our findings indicated that the three pre-treatment methods have different degrees of effectiveness in enhancing the physicochemical properties of hydrochar, with the most notable improvements were observed in MHC. We also observed that FHC significantly ($P<0.05$) inhibited germination, while MHC showed the highest germination rate, highlighting its potential as a soil amendment. The improvement in the physicochemical properties of MHC and the reduction in its phytotoxicity can be attributed to microbial degradation. However, it is important to note that the modified hydrochars still have inhibitory effects on seed germination and root development. In summary, our study suggests that pre-treated hydrochar, especially hydrochar under microbial aging, has considerable potential as a horticultural substrate.

Future research suggestions are as follows

1.Further studies should use X-ray diffraction (XRD) and thermogravimetric analyzer (TGA) to analyze the properties of hydrochar.

2.To completely eliminate the inhibitory effect, further optimization of the aging conditions is necessary.

3.Plant growth experiments are needed to validate the impact of modified hydrochar on plant growth.

Chapter 4 Growth Response of Kale to Hydrochar-Amended Growing Medium

4.1 Abstract

Hydrochar derived from hydrothermal carbonization (HTC) has received considerable attention as a soil amendment recently. However, hydrochar has been observed to have an inhibitory effect on plant growth. In this chapter, successive growth experiments were conducted to evaluate the suitability of fresh hydrochar (FHC), water-washed hydrochar (WHC), microbial-aged hydrochar (MHC) and freezing-thawing aged hydrochar (FTHC) in varying application ratios (10% and 20%) as growing medium for kale (*Brassica oleracea* var. *acephala* DC.). The control was Promix-BX peatmoss potting mix Additionally, the physiological parameters, including pH and EC, as well as the nutrient content of the growing medium were periodically evaluated. The study results revealed that hydrochar-amended growing media (FHC and treated hydrochars) had varying levels of inhibition effects on plant growth compared to the control, while 10% MHC was the least inhibitory. At the first harvest, the fresh weight of 10% MHC decreased 0.56-fold in comparison with the control, while increased by 4.5-fold and 17.6-fold in comparison with 10% FHC and 20% FHC, respectively. At the second harvest, 20% FTHC had the largest fresh weight and increased by 10.7-fold, 5.1-fold and 12.5-fold compared to the control, 10% FHC and 20% FHC, respectively. The pH and EC of the growing medium were also found increased and decreased, respectively, under MHC treatment compared to FHC treatment. Moreover, the nutrient analysis of the growing medium demonstrated that hydrochar-treated treatments contained a higher amount of nutrients and that the nutrients could be slowly released into the growing medium as supplementary fertilizer. Therefore, this study suggests that hydrochar is a promising growing medium amendment, but it needs pre-modification to reduce its inhibitory effects. Microbial aging seemed to be the best treatment for the reduction of the phytotoxicity effect. Further studies should consider adjusting aging conditions, including aging temperature and aging duration.

Keywords: hydrochar, growing media, Brassicaceae, hydrothermal carbonization, phytotoxicity

4.2 Introduction

At present, hydrochar, a soil amendment produced from biomass, has received a great deal of attention as a potential tool for improving soil quality and mitigating climate change (Hansen et al., 2016; Owsianiak et al., 2018). Hydrochar is a carbonaceous material that can be produced by hydrothermal carbonization (HTC) of biomass (Breulmann et al., 2018). In the high-temperature and pressure HTC process, hydrochar develops unique chemical and physical properties. The surface of hydrochar contains a high concentration of oxygen-containing groups (Xia et al., 2019), which suggested that hydrochar is more hydrophilic and promotes adsorption (Jin et al., 2018; Yan et al., 2018). With respect to its physical characteristics, hydrochar possesses a porous structure and high specific surface area that can facilitate the absorption-slow release of nutrients and increases soil water retention capacity (Islam et al., 2021). However, after extensive research, the effect of hydrochar as a soil amendment was not as positive as anticipated. Hydrochar has been found to negatively affect plant growth and yield. For instance, a study conducted by Puccini et al. (2018) found that hydrochar inhibited seed germination and root development in lettuce (L. *sativa* var. *Capitata*). The inhibitory effect of hydrochar was also observed in the study conducted by Gajić and Koch (2012), in which the Sugar Beet (*Beta vulgaris* L.) growth rate was reduced with the addition of hydrochar in the soil. In contrast, Reibe et al. (2015) found that hydrochar had no adverse effects on spring wheat (*Triticum aestivum* L) production. The differences could be related to the differences in the hydrochar process conditions and in the feedstocks that are used for making hydrochar (Breulmann et al., 2018).

Encouragingly, a recent study showed that while hydrochar inhibited plant growth, it did not negatively impact fruit quality (Fornes et al., 2017). Furthermore, Schimmelpfennig et al. (2014) conducted growth tests on perennial ryegrass (*Lolium perenne*) and found that the inhibitory effects of hydrochar were not permanent and only affected plant growth at an early stage. The reduction of negative effects could be attributed to the degradation of microbes upon the application of hydrochar (Ayaz et al., 2006; Wanwimolruk et al., 2015).

Based on these observations reported, it is generally believed that hydrochar would be a promising soil amendment if its negative effects can be minimized. Apart from the benefits to improve soil water retention, hydrochar has been shown to have a positive effect on the growth and abundance of microbes (Islam et al., 2021). Also, Scheifele et al. (2017) observed that hydrochar positively influenced the root nodulation of soybean (*Glycine max* L.) when used as soil amendment. Consequently, it is necessary to remove the negative effects and better display the real achievements of hydrochar materials.

Kale (*Brassica oleracea* var. *acephala* DC.), is a widely grown and consumed brassica plant with thick, flat, bluish-green leaves and thick stems (Ayaz et al., 2006; Wanwimolruk et al., 2015). In addition to its unique flavor, kale is also a rich source of antioxidants, carotenoids, fat-soluble tocopherols, ascorbic acid, and mineral nutrients. Moreover, according to Odongo et al. (2017), kale has been found to have cancer-fighting properties.

In this study, we aimed to study the effects of hydrochar as a soil amendment on the growth of kale including 1) assessment of the change in the chemical properties and nutrient content of growing medium being applied with different hydrochar materials (fresh and pre-treated spent coffee grounds based hydrochar); 2) evaluation of the growth response of kale to modified grwothing media; and determination of the optimal application rate of hydrochar.

4.3 Materials and Methods

4.3.1 Location and Materials

This study was carried out in a greenhouse at the department of Plant, Food, and Environmental Sciences, Dalhousie University Agriculture Campus, located in Truro, NS. Pro-mix BX^{TM} potting medium (Premier Horticulture Inc., Quakertown, USA) containing 75–85% sphagnum peat moss, horticultural grade perlite, vermiculite, dolomitic and calcitic limestone, was purchased from Co-op Country Store, Truro, Nova

Scotia. Kale seeds were purchased from the Halifax Seed Company (Halifax, NS, Canada). Spent coffee grounds was obtained from Tim Hortons in Truro, NS and was used to make hydrochar. Fresh hydrochar (FHC) was prepared through a HTC process. Three treated hydrochar materials include water-washed hydrochar (WHC), microbial aged hydrochar (MHC) and freezing-thawing aged hydrochar (FTHC) and were used in kael growth. The methods of fresh hydrochar production and aging procedure were described in Chapter 3.

4.3.2 Experimental design and plant growth conditions

Two growth tests were carried out successively and laid out in a completely randomized design (CRD). This growth test involved 9 treatments with four replications, four types hydrochars mixed with pro-mix BX™, in a 10:90 and 20:80 ratio, respectively. The control treatment contains Pro-mix BX™ alone. In the following second growth trials, the growing medium was kept repeat use. Seeds of kale were planted in a plastic tray and allowed to grow for four weeks before being transplanted. After four weeks, seedlings of kale were transplanted into plastic pots (one plant per pot) with a diameter of 25.4 cm (1 L volume). The cultivation period for kale plants was four weeks. During the growth period, the growing medium was watered regularly with 200 mL tap water to keep moist every two or three days, depending on the weather conditions. The greenhouse was maintained at average 24°C/20°C day and night, with a relative humidity of 71%, using a 600-watt HS2000 high pressure sodium lamp with NAH600.579 ballast (P.L. Light Systems, Beamsville, ON, Canada) for additional lighting.

4.3.3 Physicochemical properties and nutrient analysis of growing medium

The pH and electrical conductivity (EC) of growing medium were measured periodically using a multi-purpose pH meter (EC 500 ExStik II S/N 252957, EXTECH Instrument, Nashua, New Hampshire, USA). Briefly, pH and EC were measured three times during the experiment: on the first day, four weeks later, and eight weeks later. 100 g of each growing medium was taken out from the middle part of pot using a spoon. The ratio of

distilled water and growing medium was set at 5:1 (v/v). All determinations were replicated three times. In addition, the samples of growing medium were sent to Nova Scotia Department of Agriculture (NSDA) Laboratory Services, Truro, NS for nutrient analysis. The method of the AOAC-968.08 inductively coupled plasma mass spectrometer (ICP-MS) was used to determine the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), chlorine (Cl), sulphate (SO_4^{2-}) and aluminum (Al).

4.3.4 Plant growth analysis

Leaf elongation of kale was examined with a 30 cm metal ruler in first 18 days. A string label was attached to the youngest leaf of the plant (8-10 mm), every three days, the length of the leaf was measured using a 30 cm ruler. At the end of the growth trial, plant height (cm) was measured using a metal ruler from the longest leaf tip to the collar of the stem and the number of leaves was also counted. Stem diameter (mm) was measured with a Vernier calipers (Mastercraft®, Ontario, Canada). The fresh weight (g) of plants was measured using an electronic MXX-412 scale with a precision of 0.01 g (Denver Instrument Company, CO, USA).

The leaf chlorophyll content measurements were measured on three leaves of plants with a portable SPAD-502 chlorophyll meter (Spectrum Technologies Inc., Aurora, Ill., USA) prior to harvest. Chlorophyll fluorescence parameters were assessed using a portable OS30p+ Chlorophyll fluorometer (Opti-Science Inc., Hudson, NH, USA) adapted with a leaf-clip holder FL-DC clips (Opti-Sciences Inc.). First, the clips were attached on the middle part of the leaves 25 min for dark adaption. Following darkness adaptation, opened the window of the clips and the chlorophyll fluorescence indices were measured on the fluorometer. Three leaves per plant were recorded in this study. The Chlorophyll fluorescence indices were used to determine the maximum efficiency of photosystem II (Fv/Fm) thus reflecting the stress level in plants, here Fv denotes the ratio of variable fluorescence, Fm, the maximum fluorescence, Fo, the minimal fluorescence; and Fv/Fo giving the potential photosynthetic capacity. The measurement of leaf gas exchange indices (assimilation rate (A), stomatal conductance (gs), transpiration rate (E) and

intercellular CO_2 concentration (Ci)) was conducted using a LCi portable photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, England). During measurement, the chamber was fixed onto the leaf surface for 3 min, and approximately 4–6 cm^2 of leaf area (per leaf) were enclosed, while avoiding attaching the main leaf veins. Leaf gas exchange measurements were determined on the three leaves per plant between 9 AM to 1 PM on clear days.

4.3.5 Statistical analyses

All statistical analyses were performed using Minitab for Mac version 19.2020.1.0 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) was used to determine the significant difference at $P \leq 0.05$ followed by Tukey's tests to compare and assess the mean values. All graphs were generated using the software of GraphPad Prism for Mac version 9.3.1 (350) (GraphPad Inc., San Diego, CA, USA) and Origin Pro version 9.0 (Northampton, Massachusetts, USA), respectively.

4.4 Results

4.4.1 pH and EC of different growing mediums in three periods

Table 4.1 shows the pH and EC values of the growing medium for various treatments at different culture periods. A significant difference ($P<0.05$) in pH was observed at the beginning of the growth test. Notably, pH was the largest in control, and the trend of pH value was as follows: control > 20% MHC > 20 FTHC > 10% MHC > 10 FTHC > 10% WHC > 20% WHC > 10% FHC > 20% FHC. Nevertheless, after the first trial, no significant difference in pH was observed among the different growing mediums ($P>0.05$). It is worth noting that the pH of 10% FHC and 20% FHC increased by 17.9% and 22.6%, respectively, after a 4-week growth period compared to the initial period. As the result of the final measurement, there was no significant ($P>0.05$) difference in the pH of the growing mediums.

Table 4.1. pH and electrical conductivity (EC; μS/cm-1) of growing medium at different culture periods (the initial, the after first trial and the final).

Growing medium	pH			EC		
	Initial	After first trial	Final	Initial	After first trial	Final
Control	6.5 a	6.5 a	6.6 a	786.0 e	323.3 c	334.7 c
10% FHC	5.6 d	6.6 a	6.6 a	1487.7 b	466.7 b	615.3 b
20% FHC	5.3 e	6.5 a	6.6 a	1808.0 a	593.3 a	605.3 b
10% WHC	6.2 bc	6.4 a	6.5 a	788.7 e	312.0 c	576.3 b
20% WHC	6.0 c	6.4 a	6.4 a	791.7 e	313.7 c	590.7 b
10% MHC	6.3 ab	6.5 a	6.6 a	911.3 e	472.7 b	614.0 b
20% MHC	6.5 ab	6.5 a	6.6 a	1210.7 c	477.0 b	785.0 a
10% FTHC	6.3 ab	6.6 a	6.5 a	1063.7 d	453.0 b	817.7 a
20% FTHC	6.4 ab	6.5 a	6.4 a	1298.7 c	463.3 b	798.7 a
P-Value	0.000	0.107	0.107	0.000	0.000	0.000

Note: Control, promix alone; FHC, fresh hydrochar; WHC, water-washed hydrochar; MHC, microbial aged hydrochar; FTHC, freezing-thawing aged hydrochar. Different lower-case letters indicate statistical differences between different growing media at $P \leq 0.05$ (Tukey test).

In terms of EC, the addition of different hydrochar materials resulted in a significant ($P<0.05$) increase in EC of the growing mediums, as shown in Table 4.1 The highest EC was observed in the treatment with 20% FHC, while the lowest was in the control. After four weeks, all treatments still showed a significant ($P<0.05$) difference in EC, with varying degrees of decrease. EC values were decreased in the following order: 20% FHC > 20% MHC > 10% MHC > 10% FHC > 20% FTHC > 10% FTHC > control > 20% WHC > 10% WHC. However, after eight weeks, we observed that the EC of all treatments had increased compared to the results of the second measurement. The highest EC was observed in the treatment with 10% FTHC, followed by 20% FTHC, 20% MHC, and 10% MHC, with the control treatment showing the lowest EC.

4.4.2 Morpho-Physiological Response

The patterns of two plant growth trails were assessed by measuring the leaf elongation, as presented in Fig.4.1. In the first growth trial, Fig. 4.1.A shows that the addition of different hydrochar materials resulted in a significant ($P<0.05$) difference in leaf elongation. The highest leaf elongation rate was observed in control treatment, obviously an order of magnitude higher than other treatments. Followed by 10% MHC, 20% MHC, 10% FTHC, 20% FTHC, 10% FHC, 20% FHC and 10% WHC. The 20% WHC treatment exhibited the lowest leaf elongation. However, in the second growth trial (Fig. 4.1.B), the leaf elongation rate of all treatments changed considerably compared to the first growth trial. The highest leaf elongation was obtained in the treatment with 20% FTHC, followed by 10% FTHC, 20% MHC, control, 10% MHC, 20% FHC and 10% WHC. 20% WHC had the lowest leaf elongation.

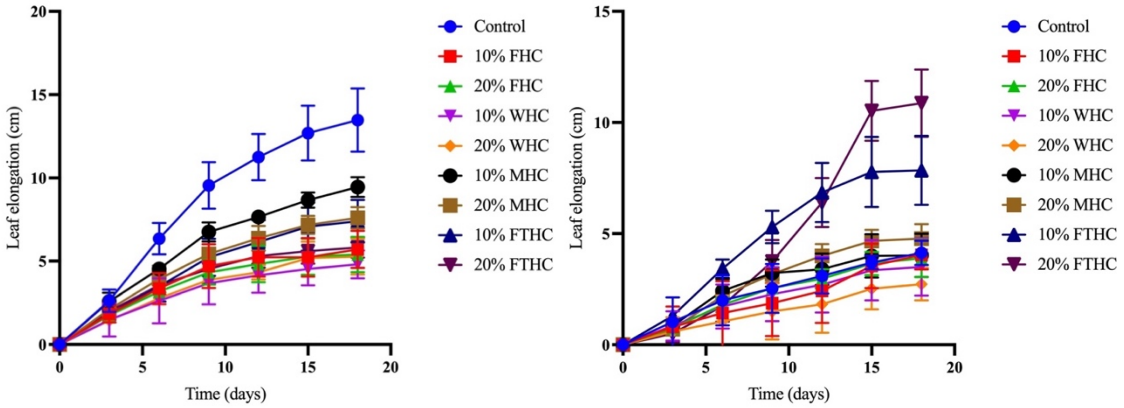
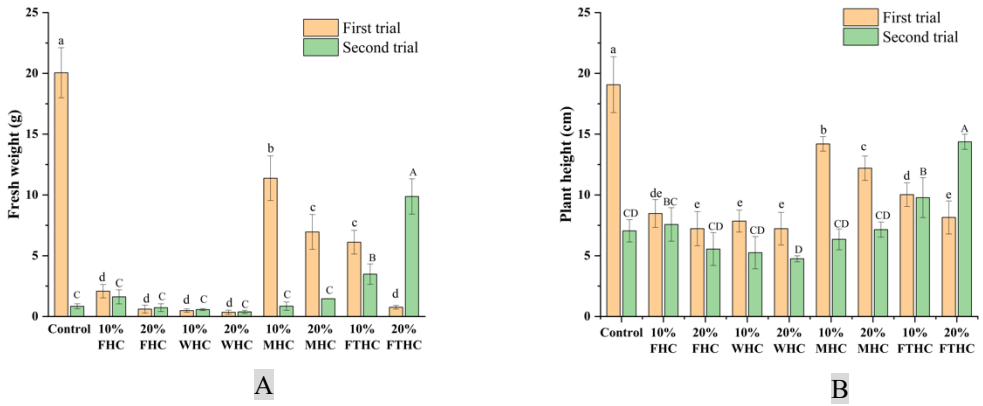


Fig. 4.1. Leaf elongation of kale grown in the different hydrochar amended growing medium. Upper-case letters indicate the first growth trial (A) and the second growth trial (B), respectively. The error bars on the graph represent standard deviation of the means.

As can be seen in Figs 4.2.A-D, the addition of hydrochars had a significant ($P < 0.05$) impact on the morphological response of plants. In the first growth trial, control plants exhibited the largest values of fresh weight, plant height, stem diameter, and number of leaves, at 20.1 g, 19.1 cm, 6.3 mm, and 12 leaves, respectively. Among all hydrochar-amended treatments, 10% MHC resulted in the best growth response. Compared to 10% FHC, 10% MHC increased the fresh weight, plant height, stem diameter, and number of leaves by 4.46-fold, 0.68-fold, 0.68-fold, and 1.9-fold, respectively.



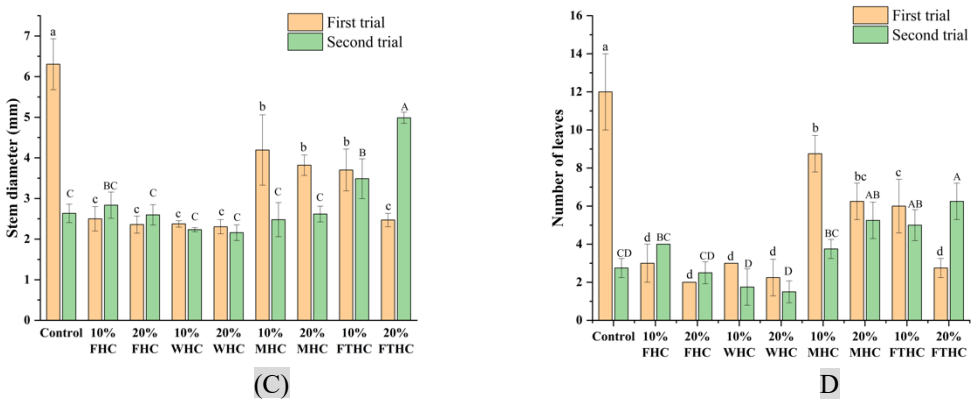


Fig. 4.2. Kale morphological response as affected by different hydrochar amended growing mediums. (A), fresh weight; (B), plant height; (C) stem diameter; (D), number of leaves. Different lowercase and uppercase alphabetical letters indicate the significant difference ($P<0.05$) among the treatments at the first and the second trial, respectively. Vertical lines on bars represent standard deviation of the means.

In second growth trial, plant morphological parameters changed considerably among treatments compared to the first trial, as shown in Figs 4.2.A-D. The highest values of plant morphological parameters were observed in 20% FTHC treatment. Specifically, in comparison to the first trial, the fresh weight, plant height, stem diameter, and the number of leaves of FTHC increased by 12-fold, 0.76-fold, 1-fold, and 1.3-fold, respectively. In addition, it should be pointed out that the application rate had a significant ($P<0.05$) effect on plant morphology values, which performed better at low concentrations (10%) in the first trial. In contrast, in the second trial, MHC and FTHC were applied at a 20% rate and showed higher morphology values. Furthermore, it is also worth noting that WHC exhibited the lowest growth performance among all treatments in both trials.

The physiological responses of kale to different hydrochars applied in two growth trials are shown in Tables 4.4 and 4.5. Due to poor growth performance, we were unable to collect physiological parameters for the treatments with 20% FHC, 10% WHC, 20% WHC, and 20% FTHC in the first growth trial. These detectable treatments, significant differences in chlorophyll fluorescence were found for chlorophyllare listed fluorescence (Table 4.4). The highest Fv/Fo and Fv/Fm were observed in 10% FTHC treatment and the lowest values were recorded in 20% MHC treatment. There was also a significant difference in SPAD values, with the largest value obtained in 10% MHC treatment and

the least value found in 10% FHC treatment. Moreover, Photosynthesis rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 were significantly ($P<0.05$) affected by treatments the addition of hydrochar in growing medium (Table 4.4). The greatest transpiration rate (E) and stomatal conductance (gs) were observed in 10% FTHC treatment, while the control treatment had the highest photosynthetic rate and the largest sub-stomatal CO_2 in 20% MHC treatment.

Table 4.4. Effects of applying different hydrochars on physiological parameters of kale in first growth trial.

Treatment	Fv/Fo	Fv/Fm	SPAD	A ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mol\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ mol^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)
Control	4.07±0.46 ab	0.80±0.02 ab	44.57±3.61	6.53±1.77 a	3.60±1.05 ab	328.83±30.06 c	0.19±0.11 ab
10% FHC	4.25±0.33 a	0.81±0.01 a	39.03±5.55	4.77±2.51 ab	3.22±0.65 ab	345.17±33.18 bc	0.14±0.04 ab
10% MHC	4.25±0.37 a	0.81±0.01 a	45.43±2.19	3.53±1.24 bc	3.05±0.70 ab	359.08±15.08 ab	0.14±0.05 ab
20% MHC	3.88±0.22 b	0.79±0.01 b	44.19±2.31	2.23±1.01 c	2.63±1.10 b	375.50±28.27 a	0.11±0.05 b
10% FTHC	4.29±0.25 a	0.81±0.01 a	44.08±3.56	6.09±1.51 a	4.00±0.93 a	348.42±14.03 abc	0.21±0.07 a
P-Value	0.021	0.026	0.000	0.000	0.006	0.001	0.005

Note: Fo: minimum fluorescence; Fm: maximum fluorescence; Fv: variable fluorescence; Fv/Fm: maximum efficiency of photosystem II; Fv/Fo: potential photosynthetic capacity; A: photosynthetic rate; E: transpiration rate; gs: stomatal conductance; Ci: sub-stomatal CO_2. NA: data not available. Values are expressed as means±deviations. Different lower-case letters between same columns indicate significant difference between the results ($p<0.05$) according to Turkey's test.

Table 4.5. Effects of applying different hydrochars on physiological parameters of kale in the second growth trial.

Treatment	Fv/Fo	Fv/Fm	SPAD	A ($\mu mol\ m^{-2}\ s^{-1}$)	E ($mol\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ mol^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)
10% FHC	2.90±0.74 a	0.73±0.06 a	37.74±6.57 b	1.31±0.66 b	1.77±0.61 c	313.00±30.98 ab	0.03±0.01 b
20% MHC	3.23±0.50 a	0.76±0.03 a	33.90±6.09 b	3.45±1.71 a	3.08±0.91 b	288.58±24.97 b	0.06±0.02 ab
10% FTHC	2.90±0.49 a	0.74±0.03 a	37.51±5.41 b	2.245±1.43 ab	5.56±1.85 a	344.8±93.2 a	0.09±0.04 a
20% FTHC	3.39±0.26 a	0.77±0.01 a	44.82±2.63 a	3.49±2.33 a	3.76±2.08 b	301.17±20.52 b	0.10±0.09 a
P-Value	0.017	0.028	0.000	0.005	0.006	0.001	0.005

Note: Fo: minimum fluorescence; Fm: maximum fluorescence; Fv: variable fluorescence; Fv/Fm: maximum efficiency of photosystem II; Fv/Fo: potential photosynthetic capacity; A: photosynthetic rate; E: transpiration rate; gs: stomatal conductance; Ci: sub-stomatal CO_2. NA: data not available. Values are expressed as means±deviations. Different lower-case letters between same columns indicate significant difference between the results ($p<0.05$) according to Turkey's test.

In the second growth trial, similarly, physiological parameters were not available for treatments of control, 20% FHC, 10% WHC, 20% WHC, and 10% MHC. Leaf chlorophyll fluorescence indices were significantly ($P<0.05$) higher with 20% FTHC treatment than other treatments. Additionally, 20% FTHC treatment had the highest SPAD value, followed by 10% FTHC, and the lowest was observed in 10% MHC treatment (Table 4.5). All treatments had significant ($P<0.05$) effects on leaf gas exchange. The largest values for photosynthetic rate (A) and stomatal conductance (gs) were observed in 20% FTHC treatment. The highest values of transpiration rate (E) and sub-stomatal CO_2 (Ci) were recorded in 10% FTHC treatment (Table 4.5). Additionally, it is worth mentioning that compared to the first trial, the maximum values for almost all parameters were slightly lower in the second trial.

4.4.3 Nutrient analysis of growing medium and leaf tissues

Table 4.6 shows the nutrient content of different growing mediums over three periods. A considerable amount of nutrients was found in each growing medium, and the nutrient content differed greatly between media. At the beginning of the growth trial, the application of hydrochar led to an increase in nitrogen (N) content, with the highest N obtained in 20% FTHC treatment compared to the control. Moreover, 20% FHC contained higher amounts of Ca, K, Mg, P, Fe, and Mn, respectively, by approximately 2.4-fold, 2.4-fold, 2.6-fold, 3.0-fold, 0.2-fold, and 1.6-fold, as compared to the control. Treatment with 10% MHC exhibited comparable amounts of Na, Cl, and Zn, which increased by approximately 0.7-fold, 0.30-fold, and 0.08-fold, respectively, compared to the control. However, after the first growth trial, a dramatic decrease was observed in the nutrient content of each growing medium except for N. The greatest amount of N was still found in 20% FTHC treatment, which also had the highest K content. The highest amounts of Ca, Mg, and Cl were found in 10% MHC, while 10% FHC had largest P amount. The control group had the highest amount of Na. In comparison to the second period nutrient measurement, the third nutrient analysis of all hydrochar-amended growing media showed a significant increase in nutrient content, except for N. The

highest N and Na were found in treatment 20% MHC, while 20% FTHC treatment contained higher amounts of Ca, K, Mg, P, Fe, and Zn.

Table 4.7 and Table 4.8 show the nutrient content of leaf tissues. At the first harvest, only the leaf mineral content data for the treatments of control, 10% MHC, 20% MHC, and 10% FTHC were available. Compared to the control, these treatments resulted in an increase in almost all nutrient elements. Specifically, 20% MHC had the highest amount of Ca, K, Mg, Na, B, and Fe, while the highest value of N, P, and Zn was found in 10% FTHC. However, only 10% FTHC and 20% FTHC had sufficient samples for nutrient analyses at the second harvest. A higher amount of Ca, Mg, P, B and Na were observed in 10% FTHC, while 20% FTHC had higher amounts of N, Cu, Fe, Zn, and Mn.

Table 4.6. Mineral nutrient composition of the growing media in three periods

Nutrients	**Treatment**								
Initial	Control	10% FHC	20% FHC	10% WHC	20% WHC	10% MHC	20% MHC	10% FTHC	20% FTHC
N (%)	0.53	1.04	1.38	1.04	1.26	1.17	1.55	1.16	1.62
Ca (mg/L)	79.89	191.92	269.81	88.86	77.44	103.74	62.35	62.37	61.31
K (mg/L)	73.01	168.67	224.59	88.63	81.9	175.74	132.95	104.89	135.09
Mg (mg/L)	19.17	49.31	69.5	22.24	20.12	30.04	17.67	16.83	18.84
P (mg/L)	13.72	40.05	54.52	16.02	11.43	34.87	27.55	23.78	28.49
Na (mg/L)	18.05	26.3	26.06	19.95	17.93	29.91	23.26	20.55	22.94
Cl (mg/L)	29	29	29	29	29	38	38	29	29
Fe (mg/L)	0.75	0.82	0.89	0.85	0.84	0.87	0.69	0.66	0.5
Zn (mg/L)	1.46	1.52	1.19	1.52	1.27	1.58	0.68	0.99	0.8
After first trial									
N (%)	0.52	1.05	1.19	1.13	1.29	1.2	1.5	1.06	1.55
Ca (mg/L)	33.6	27.3	19.76	19.7	20.76	33.85	29.76	20.24	22.55
K (mg/L)	9.71	67.84	47.71	41.62	42.07	52.08	75.77	43.89	73.47
Mg (mg/L)	8.35	6.88	5.25	5.05	5.51	8.37	7.66	4.89	6.06
P (mg/L)	8.38	18.44	15.67	13.79	13.63	17.2	17.5	13.59	22.1
Na (mg/L)	29.99	19	13.85	13.67	14.65	26.74	22.08	16.79	16.28
Cl (mg/L)	29	29	29	29	29	48	29	29	29
Fe (mg/L)	0.16	0.09	0.07	0.07	0.07	0.21	0.25	0.17	0.15
Zn (mg/L)	0.03	0.04	0.03	0.03	0.04	0.05	0.06	0.04	0.06

Final									
N (%)	0.58	1.05	1.36	1.12	1.43	1.21	1.47	1.13	1.39
Ca (mg/L)	30.52	40.43	40.44	39.25	46.36	44.34	60.92	62.44	62.83
K (mg/L)	8.13	73.21	72.98	62.95	67.46	60.51	99.81	91.4	130.13
Mg (mg/L)	7.91	10.23	10.86	10.44	12.66	11.94	17.42	16.73	18.19
P (mg/L)	4.52	14.55	14.12	13.01	15.03	12.31	19.34	22.68	37.14
Na (mg/L)	41.34	37.58	34.45	35.6	39.25	43.03	49.85	48.53	25.27
Cl (mg/L)	38	38	38	48	48	48	48	57	38
Fe (mg/L)	0.06	0.17	0.1	0.08	0.07	0.11	0.14	0.25	0.45
Zn (mg/L)	0.02	0.04	0.04	0.03	0.04	0.06	0.07	0.49	0.93

Note: N, nitrogen; Ca, calcium; K, potassium; P, phosphorus; Mg, magnesium; Na, sodium; Cl, chloride; Al, Aluminum; B, boron; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; Control, Promix-BX only; FHC, fresh hydrochar; WHC, water washed hydrochar; MHC, microbial aged hydrochar; FTHC, freezing thawing aged hydrochar.

Table 4.7. Mineral nutrient composition of the leaf tissue in first growth trial.

Nutrients	Control	10% MHC	20% MHC	10% FTHC
N (%)	1.85	2.41	2.73	3.05
Ca (%)	2.18	2.44	2.67	2.62
K (%)	3.04	4.40	6.46	6.26
Mg (%)	0.38	0.44	0.51	0.50
P (%)	0.42	0.99	1.19	1.21
Na (%)	0.08	0.05	0.08	0.08
B (ppm)	20.72	32.79	38.89	37.92
Cu (ppm)	6.06	12.88	12.83	10.89
Fe (ppm)	52.56	55.83	59.44	57.32
Mn (ppm)	24.04	45.82	73.44	53.48
Zn (ppm)	65.85	101.78	150.74	156.94

Table 4.8. Mineral nutrient composition of the leaf tissue in second growth trial.

Nutrients	10% FTHC	20% FTHC
N (%)	0.84	1.18
Ca (%)	2.28	1.75
K (%)	2.36	3.25
Mg (%)	0.43	0.35
P (%)	0.86	0.80
Na (%)	0.042	0.03
B (ppm)	24.09	23.18
Cu (ppm)	5.21	7.13
Fe (ppm)	35.57	40.62
Mn (ppm)	73.04	106.60
Zn (ppm)	66.76	83.00

Note: N, nitrogen; Ca, calcium; K, potassium; P, phosphorus; Mg, magnesium; Na, sodium; B, boron; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc. Control, Promix-BX only; MHC, microbial aged hydrochar; FTHC, freezing thawing aged hydrochar.

4.5 Discussion

4.5.1 Possible explanations of pH and EC changes

The pH of soil is one of the most important soil chemical parameters, and numerous studies have demonstrated that pH affects nutrient uptake during plant growth (de Jager and Giani, 2021; Soti et al., 2015). Msimbira and Smith (2020) revealed that the optimal pH range for plant growth is between 5.5 and 6.5. In this study, we observed that the addition of FHC at application rates of 10% and 20% had an immediate effect on the pH of the growing medium, lowering pH values. This suggests that the addition of FHC had an initial acidifying effect on the growing medium, which may negatively influence plant growth. In the second growing media chemical measurement, the increase in pH in the 10% and 20% FHC treatments was observed which is consistent with previous studies (Bargmann et al., 2014; Melo et al., 2017; Rillig et al., 2010). Rillig et al. (2010) suggested that microorganisms may be responsible for the increase in soil pH, and we may speculate that a similar mechanism may have caused the observed pH to increase in this study. Future studies should investigate the long-term effects of hydrochar on soil/growing medium pH and plant growth to better understand its potential as a soil amendment.

The addition of FHC significantly increased the electrical conductivity (EC) of the growing medium, which is consistent with the findings of Belda et al. (2016). Additionally, the research conducted by Puccini et al. (2018) aligns with our findings, as they observed a decline in the EC of hydrochar by 68% and 47% due to washing and aging, respectively. To date, very little study has been conducted on the effects of hydrochar on the EC of growing media throughout the growth period. According to Agegnehu et al. (2016), hydrochar application did not significantly change the EC during the growth period. However, Arthur and Ahmed (2017) found that the addition of biochar increased soil EC from 123 S cm^{-1} to 141 S cm^{-1} over 15 months. Additionally, Ro et al. (2016) demonstrated that the EC of hydrochar-amended growth medium decreased from 520 dS m^{-1} to 457 dS m^{-1} after repeated assessments of leachate quality. In this study, we

observed that the EC values varied at different growth stages, this phenomenon may be related to the porous structure of the hydrochar (Shao et al., 2020), which leads to changes in the EC value due to the absorption/release of soluble salts on the surface of the hydrochar, further research is needed to confirm the mechanism responsible for these variations.

4.5.2 Suitability of various hydrochars for kale production

In this study, the results of kale growth revealed that the 10% FHC and 20% FHC treatments had a considerable negative impact on the growth rate and morphology of kale plant compared to the control, indicating a phytotoxic effect of hydrochar. Similar findings were observed in previous studies conducted by Bargmann et al. (2013), Busch et al. (2013) and Cervera-Mata et al. (2021). These findings suggested that FHC is not a suitable soil amendment for immediate incorporation into the growth medium. However, Schimmelpfennig et al. (2014) observed that pre-incubation of the soil and HTC-slurry for three months considerably reduced the inhibitory effect on ryegrass (*Lolium perenne*). Similarly, Busch et al. (2012) reported that the growth of barley was inhibited by hydrochar during the first growth trial, but positive results were observed during the second growth trial. However, our study did not observe a substantial improvement in kale growth compared to the first trial. This may be due to the following reasons: firstly, as compared to the three-month incubation period in the study by Schimmelpfennig et al. (2014), we may need more time to incubate the hydrochar-amended growth media. Secondly, harmful substances present in hydrochar can be degraded by microorganisms during long-term natural soil aging (Bargmann et al., 2013; Wang et al., 2021). Therefore, we speculate that Promix-BX does not contain as many microbes as soil does. Hence, since fresh hydrochar derived from spent coffee grounds inhibits plant growth, it is suggested that it should be pretreated and aged long enough before being applied as a soil amendment.

The present study found that 10% WHC and 20% WHC were not suitable for growing kale. Particularly, the 20% WHC treatment had the poorest growth performance, even

worse than that of 20% FHC. These results are in contrast to prior studies that reported the positive effects of WHC on plant growth (Chen et al., 2021; Wu et al., 2021; Yu et al., 2019). Unfortunately, this result has not previously been described. We could speculate that the observed negative effect of WHC on plant growth may be attributed to the removal of ash content and nutrients from the hydrochar's surface through washing. More future studies are needed to explain this phenomenon.

Our results indicated that the microbial aging method is effective in reducing the phytotoxicity of hydrochar. This finding was consistent with previous studies by Gul and Whalen (2016) and Yu et al. (2019). It may be possible to attribute the reduction in phytotoxicity to the microbial degradation. However, our study found that the plant growth response of MHC differed slightly from that of Yu et al. (2019), as the yield and growth response of kale was not superior to that of the control. This suggests that phytotoxic components still existed in MHC and had not been eliminated entirely via microbial aging method. This study observed the reduction in phytotoxicity via the microbial aging method, however, the potentially harmful compounds of hydrochar derived from coffee grounds were not confirmed. It is necessary to do the further analysis of hydrochar's chemical compounds, particularly phenols, PAHs, and organic acids. Moreover, extensive previous research has shown that hydrochar can serve as soil amendment and slow-release nutrients to soil for plant growth (Gronwald et al., 2016; Wu et al., 2021). In this study, the superior growth performance of MHC treatments over FHC and WHC treatments in the second growth trial may also be attributed to the slow-release of nutrients by MHC. However, the plant growth performance of MHC in the second trial was not as excellent as in the first growth trial, possibly due to the plants in the first growth trial absorbed more nutrients from the growth medium.

FTHC exhibited a lower phytotoxicity than FHC. However, few investigations on freezing-thawing have been undertaken to reduce the phytotoxicity of hydrochar. During the freezing-thawing aging process, the soluble organic components of hydrochar may be eliminated by the microorganisms which may result in a decreased phytotoxicity (Korai at al., 2018). In the second growth trial, 20% FTHC had the best growth performance

among all treatments, which could be attributed to the following reasons: (1) as previously indicated, microorganisms in the growing media may reduce the phytotoxicity of FTHC, which becomes less phytotoxic after the first growth trial. (2) treatment 20% FTHC slowly released the absorbed nutrients as a medium-term fertilizer.

4.5.3 Effect of various hydrochars on plant physiological parameters

This study found that hydrochar, pre-treated or not still inhibited plant growth. Nevertheless, hydrochar still stands out in terms of plant physiological indices. Chlorophyll fluorescence is often used to measure the functional levels of photosynthesis and as an indicator of plant health (Lin et al., 2021). Thuille et al. (2015) reported that the chlorophyll fluorescence content of corn (*Zea mays*) was increased in hydrochar-amended soil, which is consistent with the results of this study. In the first growth trial, we observed that kale grown in soil amended by 10% FHC, 10% MHC, and 10% FTHC had higher values of Fv/Fo and Fv/Fm compared to the control. However, the chlorophyll fluorescence content in 20% MHC was lower than that in the control, indicating that the application rate of hydrochar can affect chlorophyll fluorescence content. In a previous study, hydrochar produced from forest waste has been shown to negatively affect the SPAD values of calendula (*calendula officinalis* cv.) and Petunia (*Petunia hybrida* cv.) at 10%, 25% and 50% application rate (Fornes and Belda, 2018), which is consistent with the results of this study that 10% FHC treatment greatly decreased SPAD. However, we also observed that SPAD was increased in 10% MHC, 20% MHC, and 10% FTHC compared to 10% FHC. This suggests that microbial aging and freezing-thawing aging are two ways that may effectively mitigate the negative impact of hydrochar on plant SPAD values.

4.5.4 Effect of different hydrochar on nutrients contents in leaf tissues and growing medium

The mineral elements are essential for plant growth, and nutrient deficiencies can result in stunted growth and decreased yields (Fageria and Moreira, 2011).The first growing

media chemical measurement revealed that hydrochar is rich in various nutrients, particularly 20% FHC. However, we also observed a decrease in nutrient content in 10% WHC and 20% WHC, indicating that water washing not only removed soluble organic matter but also nutrients from hydrochar. It should be noted that while MHC and FTHC contained more nutrients than the control, their nutrient content was lower than FHC. A possible explanation for this could be that during the aging process the nutrients may have been consumed by the microbes (Shi et al., 2021). In the second measurement, a considerable decrease in nutrient content was observed in all treatments. The following reasons can be attributed to the decrease: (1) nutrients were absorbed by plants for growth; (2) as a result of hydrochar adsorption, nutrients were absorbed; (3) leaching caused by watering led to nutrient loss. Our third nutrient measurement of the growth media revealed that all hydrochar-amended growing medium, except for control, showed varying degrees of improvement in nutrient content, providing further evidence that nutrients can be slowly released by hydrochar. Additionally, all hydrochar-amended growing mediums had a higher N content than the control treatment, which is consistent with the findings of Chu et al. (2020) that paddy soil retention of N increased with hydrochar application. Meanwhile, Gajić and Koch, (2012) reported that N immobilization occurred when hydrochar was added to soil, which results in a low level of N availability to plants. In this study, N immobilization was not measured. However, we noticed that the hydrochar-treated plant tissues had a high level of N, therefore, we assume that N immobilization played a minor role in the study. With respect to the nutrient content of leaf tissues, 10% MHC, 20% MHC and 10% FTHC treatments were observed to have a higher amount of nutrient content compared to control, which might be related to the high nutrient content offered by hydrochars.

4.6 Conclusion and Future Outlook

This kale growth experiment revealed that application of FHC significantly inhibited the growth of kale. However, we found that the microbial aging method was efficient in reducing the inhibitory effect of hydrochar on kale, especially at low the application rate of 10%, but it did not completely remove the inhibitory effect as evidenced by Yu et al. (2019) compared to control. The freezing-thawing aging method also demonstrated its

ability of reducing phytotoxicity of hydrochar, but it was not as efficient as microbial aging method. In addition, we discovered that the addition of hydrochar could provide more nutrients for plant growth. MHC and FTHC demonstrated that they can deliver nutrients to plants during the growth period via nutrient absorption and slow-release. In future research, it will be necessary to conduct additional chemical analyses on hydrochar to identify the damaging components that are harmful to plant growth. Furthermore, the conditions of aging can be altered to completely eliminate hydrochar's phytotoxicity.

Chapter 5 Recommendation to Future Work

In this experiment, we have demonstrated the inhibitory effect of FHC on plants and the positive impact of the modification methods on the physicochemical properties of hydrochar, seed germination, and plant growth. To better understand hydrochar and make better use of it in agriculture, the followingwork are recommended for the future.

Firstly, it is necessary to analyze the nutrient composition and ash content of hydrochar in future studies to better understand its physiochemical properties. In addition, this experiment used kale as the test plant to verify the phytotoxicity of different hydrochars, but the specific toxic components have not been identified. Therefore, the potential harmful components in hydrochar such as phenols, organic acids, and heavy metals should be identified and quantified.

Regarding the modification method, we found that WHC had a negative impact on plant growth, contrary to previous research results. Therefore, in future studies, we need to retest the effect of washed hydrochar on plant growth and try using longer washing times and multiple water changes for washing. For MHC and FTHC, different aging times and freeze-thaw durations can also be attempted.

The effect of hydrochar on the biological characteristics of growth medium was not analyzed in this experiment. Therefore, future studies should analyze the impact of hydrochar on the abundance and diversity of bacteria and fungi in the growth media.

This experiment was conducted in a greenhouse. To explore the impact of hydrochar on soil and crop growth in natural environments, it is necessary to conduct field studies. Performance of hydrochar as a soil amendment is highly associated with the parent biomass that hydrochar is produced from, soil properties and the species of plants. It is recommended to extend testing on various hydrochar and different plants.

Chapter 6 Conclusion

In modern agriculture, protecting and improving soil health is important to achieving food security and sustainable agricultural development. Hydrochar derived from HTC of biomass waste is considered as a promising soil amendment due to its unique physiochemical properties and environmentally friendly production process. However, hydrochar could have phytotoxic effects that inhibit plant growth. Therefore, this study particularly aims to evaluate the suitability of freshly made hydrochar and three other modified hydrochars for current agricultural practices. This research provides valuable insights for the use of hydrochar in agriculture, the effect of application of hydrochar on soil health as well as the role that hdyrochar plays in sustainable agriculture.

The research in Chapter 3 focused on the effects of pre-treatment methods i.e., water washing, microbial ageing and freeing -thawing aging on the physical and chemical properties of the resulting hydrochar, and their impacts on kale seed germination. It was observed that microbial aging was superior to water washing and freezing-thawing aging in improving hydrochar physicochemical properties. Specifically, under microbial aging, hydrochar showed a larger surface area, a significant reduction in bulk density, and a significant increase in porosity and water-holding capacity. Additionally, the pH of hydrochar increased and EC decreased in addition to increased oxygen-containing functional groups on its surface after microbial aging. Seed germination test revealed that FHC remarkably inhibited kale seed germination, while all three pre-treated hydrochars promoted seed germination. The MHC gave the highest germination rate. These findings suggest that pre-treatment, especially microbial aging, can improve the physiochemical properties of hydrochar and reduce its phytotoxicity, making it a potentially suitable soil amendment for plant growth.

Chapter 4 further reported the effects of four different hydrochars (i.e., fresh, water-washed, microbial aged and freezing -thawing aged hydrochars) on kale plant growth at two application rates, 10% and 20%. The results indicated that FHC significantly inhibited kale growth. However, this inhibitory effect was significantly reduced by

application of 10% microbial aged hydrochar. Except for the control, kale grown under 10% MHC amended growing medium showed the highest fresh weight, plant height, and number of leaves per plant. This suggests that microbial aging can effectively reduce phytotoxicity, but not completely as the kale plant growth was not as good as that of the control plants. The changes in the physical properties of hydrochar after microbial aging and freezing-thawing treatment can better facilitate nutrient release through increased porous structure. Plants grown in treated growing medium with MHC and FTHC had higher nutrient contents compared to the control treatment. This chapter suggests that hydrochar, especially after microbial aging, can be a potential soil amendment for promoting plant growth and nutrient supply.

In summary, this research has confirmed that hydrochar derived from HTC of coffee grounds has inhibitory effect on kale growth. However, the results also suggest that hydrochar can be used in agriculture if is properly pre-treated. Under the scope of this current study, microbial aging appears to be a promising method for reducing hydrochar's inhibitory effect on plant growth. The reduction in inhibition might be attributed to the microbial degradation.

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