# Investigate the Chemistry and Agricultural Efficacy of Hydrothermal Carbonization Processed Liquid Extracted from Willow (*Salix babylonica*) Branches, Seafood Compost, and Buckwheat (*Fagopyrum esculentum*) Green Foliage

By

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## ABSTRACT

Hydrothermal Carbonization Processed Liquid (HTCPL) has been regarded as a by-product in the HTC process. This study focused on analyzing and comparing the composition and properties of HTCPL derived from willow (*Salix babylonica*) branches, seafood compost, and buckwheat (*Fagopyrum esculentum*) green foliage processed at 180 ℃ and 200 ℃ and evaluate the role of HTCPL in agriculture. The work found that the HTCPL samples are rich in macronutrients for plants. HTCPLs also contain a small number of micronutrients required by plants. Therefore, it is preliminarily considered that the HTCPL has the potential as a bio-fertilizer. Further experiments of this work indicated that HTCPL with different concentrations did not show the promoting effect on seed germination and seedling growth. But this work also found that the HTCPL extracted from different feedstocks with appropriate concertation can be used as a bio-fertilizer to cultivate pea (*Pisum sativum* L.) baby greens.

*Keyworks: hydrothermal carbonization, bio-fertilizer, seed germination, seedling growth, baby greens*

## LIST OF ABBREVIATIONS AND SYMBOLS USED

| | |
|---|---|
| B | Boron |
| Ca | Calcium |
| cm | Centimeter |
| CS-CM | Corn-silage-and-cow-manure |
| Cu | Copper |
| EC | Electrical conductivity |
| g | Grams |
| hrs | Hours |
| HTC | Hydrothermal Carbonization |
| HTCPL | Hydrothermal Carbonization Processed Liquid |
| K | Potassium |
| Mg | Magnesium |
| N | Nitrogen |
| $NH_4^+$ | Ammonium |

| | |
|---|---|
| $NO_3^-$ | Nitrate |
| $NaNO_3$ | Sodium Nitrate |
| P | Phosphorus |
| ppm | Parts-per-million |
| psi | Pounds-per-square-inch |
| SC | Seafood compost |
| TOC | Total-organic-carbon |
| μohms | Micromhos |
| Z | Zinc |

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# Chapter 1 Introduction

## 1.1 General Introduction

Hydrothermal Carbonization Processed Liquid (HTCPL) is a waste product of hydrothermal carbonization process (HTC). HTC is a thermochemical process that converts organic materials to value-added products by heating organic biomass as feedstocks to between 180 °C and 350 °C (Saetea & Tippayawong, 2013). HTCPL was once considered a valueless by-product of hydrochar until studies found that HTCPL has the potential to promote plant growth (Sun et al., 2014; Funke, 2015; Yao et al., 2016). Previous studies have shown that the kind of organic biomass, heating rate, residence time, temperature, and particle size during the HTC process influence the composition and property of HTCPL (Saetea & Tippayawong, 2013; Sun et al., 2014; Funke, 2015). Typically, the pH value of HTCPL is unstable and varies according to the source of biomass, heating temperature and other factors during the HTC process (Sun et al., 2014). Many different organic biomasses have been used to produce HTCPL such as plant biomass, composts, and even sewage sludge (Funke, 2015; Idowu et al., 2017; Sun et al., 2014; Yao et al., 2016). Although some studies have suggested that HTCPL has the potential to affect plant growth (Yao et al., 2016), there are few documented literature information on the compositions and properties of HTCPL. Also, the effect of HTCPL obtained from different organic biomasses in agricultural production is understudied. Therefore, it is necessary to compare HTCPL extracted from different biomasses and to evaluate their efficacy on plant growth performance and development.

Lettuce (*Lactuca sativa*) is one of the best-selling vegetables in the world, and its largest producer is China (Mou, 2008). Lettuce is a good source of fibre, iron, folic acid and vitamin C (USDA, 2015). In addition, lettuce is rich in a variety of biologically active compounds that are beneficial to health and is low in calories and sodium (Baslam et al., 2013; Guedard et al. 2008). Studies have shown that lettuce is effective in anti-inflammatory, cholesterol-lowering and anti-diabetic (Carr & Frei, 1999; Lee et al., 2009; Nicole et al., 2004; Pepe et al., 2015).

Kale (*Brassica oleracea* var. *sabellica*) is a leafy vegetable with high nutritional value, which is widely planted in Europe and North America (Neugart et al. 2012). Kale is rich in flavonoids, which can effectively prevent human chronic diseases (Knekt et al. 2002; Schmidt et al. 2010). In addition, kale has abundant content of vitamin C and phenolic compounds with high antioxidant activities (Cartea et al. 2011; Jahangir et al. 2009).

Pac choi (*Brassica campestris* L. *ssp.*) also belongs to the *Brassica*-family. The rich bioactive substances in pac choi such as glucosinolates and carotenoids give pac choi a high nutritional value (Kaulmann et al., 2014; Wiesner et al., 2014). It has been proven that eating *Brassica*-family vegetables have anti-cancer effects (Day et al., 1994; Thangam et al., 2013). In China, pac choi has been eaten as a traditional food for thousands of years (Shimin et al., 2016). Due to its unique appearance and flavor, as well as high nutritional value, pac choi has gradually become popular in Western diets (Rochfort et al., 2006).

Sunflower (*Helianthus annuus* L.) is an important oil crop in the world (Delplanque, 2000; Stefansson, 2009). Sunflower seeds have high-quality protein, unsaturated fats, carbohydrates and fiber (Srilatha and Krishnakumari, 2003). In addition, sunflower seeds are rich in minerals and vitamins such as selenium, zinc, and vitamin E (USDA 2008). Compared with other oil crops, sunflower seeds have high antioxidant properties, can reduce the risk of cardiovascular diseases, and have the potential to reduce the potential for cancer (Delplanque, 2000; Srilatha and Krishnakumari, 2003; WCRF, 1997).

Pea (*Pisum sativum* L.) is one of the most common plant crops, and its usually eaten as seed (Merwad, 2018). Peas are rich in high-quality protein and also, peas are a good source of carbohydrates and microelements such as calcium and vitamin A (Merwad, 2018). In recent years, ready-to-eat pea baby greens have become more and more popular. As a kind of ready-to-eat green vegetables, pea shoots are rich in many kinds of micronutrients such as vitamin C, vitamin E, vitamin A and potassium (Santos et al., 2014). The large number of flavonoids in the leaves of pea baby greens (329 mg/100 g) can prevent chronic diseases (Santos et al., 2014).

With the improvement of living standards, people increasingly tend to explore new foods with high nutritional value and rich sensory traits such as baby greens (Baselice et al., 2017; Di Bella et al., 2020; Grahn et al., 2015). In the United States alone, the market demand for baby greens has increased five-fold in the past 20 years (Grahn et al., 2015). Baby greens are immature young crops and need nutrients and light to grow, which is usually harvested after the true leaves develop (Di Gioia et al., 2017). The growth cycles of baby greens are very short, usually between 20 and 40 days (Di Gioia et al., 2017). The main edible parts of baby greens are the baby leaves and stems, which are important for both flavor and addition to salad and wraps (Baselice et al., 2017; Di Bella et al., 2020; Grahn et al., 2015). In addition to aromatic flavors, rich colors, and textures, baby greens are rich in plant nutrients values such as ascorbic acid, carotenoids, calcium, and magnesium (Di Bella et al., 2020; Francis et al. 2012; Lenzi et al., 2019).

This project focuses on the extraction and comparison of HTCPL from three different biomass; namely, willow (*Salix babylonica*) branches, seafood compost, and buckwheat (*Fagopyrum esculentum*) green foliage at different thermochemical temperatures (180 ℃ and 200 ℃). The efficacies of the HTCPL on seed germination and seeding growth of lettuce, kale, pac choi, sunflower and pea were assessed. Also, pea baby greens response to HTCPL extracted from different biomasses were evaluated.

## 1.2 Thesis Objective and Organization

The overall objective of this thesis is to evaluate the chemistry and agricultural efficacy of HTCPL extracted from willow branches, seafood compost, and buckwheat green foliage. There are three sub-objectives here, namely, 1). compare the composition and properties of HTCPLs derived from willow branches, seafood compost and buckwheat green foliage; 2). investigate the effect of HTCPL from different biomasses on seed germination and seedling growth; 3). investigate the effect of HTCPL from different biomasses on pea baby-greens growth and mineral composition. Depending on the study of Abbey et al. (2018), seafood compost is rich in macronutrients such as nitrogen and potassium and micronutrients such as copper and manganese that significantly improve plant growth, yield and quality. A previous study has suggested that HTCPL obtained at 200 ℃ is most suitable for plant growth (Sun et al. 2014). Therefore, the general hypothesis of the research is that application of HTCPL extracted from seafood compost at 200 ℃ temperature can significantly improve seed germination, plant growth performance and quality compared to HTCPL extracted from willow and buckwheat. Three individual experiments were conducted in this project. Chapter 1 is an **Introduction** to this study, including General Introdution and Thesis Objective and Organization. Literature Review is presented in Chapter 2. Chapter 3 contains the first experiment on **Extraction and Analysis of Liquids Produced from Different Biomasses by the Hydrothermal Process and Potential**

**Estimation of Hydrothermal Carbonization Processed Liquid for Agricultural Utilization**. The aim is to compare the chemical profiles of HTCPL derived from willow branches, seafood compost, and buckwheat green foliage at different thermochemical temperatures (180 ℃ and 200 ℃) and to evaluate the utilization potential of HTCPL on plant growth and development. Chapter 4 represents the second experiment, on **Seed Germination and Seedling Growth Response to Hydrothermal Carbonization Processed Liquid Extracted from Different Biomass**. The objectives of the second experiment are to investigate the seed germination and the development of radicle (embryonic root) and hypocotyl (embryonic shoot) of the five plants (i.e., pea, sunflower, pac choi, kale, and lettuce) response to HTCPL derived from willow branches, seafood compost, and buckwheat green foliage at 200 ℃. The experiment in Chapter 4 consists of two independent experiments. The experimental procedures of Experiment 1 and Experiment 2 of Chapter 4 are similar, only treatments and objects are changed. The purpose of designing Experiment 2 is to have a deeper understanding of the impact of HTCPL on seed germination and seedling growth. It is worth mentioning that the research on Experiment 1 in Chapter 4 was submitted for publication. Chapter 5 was on **Pea Baby Greens Growth and Mineral Quality Response to Hydrothermal Carbonization Processed Liquid Extracted from Different Biomass**. The aim of the third study is to investigate the effects of HTCPL derived from willow, seafood compost, and buckwheat on peas baby leaf greens growth and mineral quality. The Overall Conclusion and Recommendation of this study is presented in Chapter 6.

# Chapter 2 Literature Review

## 2.1 Hydrothermal Carbonization Processed Liquid (HTCPL)

### 2.1.1 HTC versus HTCPL

Hydrothermal Carbonization (HTC) is a thermochemical process that converts organic materials to value-added products at moderate temperatures. The common HTC reaction temperature range is from 180 ℃ to 350 ℃ (Saetea & Tippayawong, 2013). The products of HTC are solid, liquid and a small amount of gas. The main valuable outcome of HTC is solid, hydrochar. Hydrochar has many uses, such as soil amendment, water purifier, and metal adsorbent. Hydrothermal Carbonization Processed Liquid (HTCPL) was considered as a by-product in the process of making hydrochar, which has the potential to be applied flexibly as fertilizer (Sun et al., 2014; Funke, 2015; Yao et al., 2016). Many organic biomasses can be used as feedstock for HTC such as various plants, composts, and even sewage sludge (Saetea & Tippayawong, 2013; Funke, 2015; Yao et al., 2016; Idowu et al., 2017). The different feedstocks lead to different chemical compositions and properties of HTCPL (Hellmann et al., 2011; Escala et al., 2012; Funke et al., 2013; Reza et al., 2013; Saetea & Tippayawong, 2013; Idowu et al., 2017). Also, depending on heating rate, residence time, heating temperature, and biomass particle size during the HTC process, the chemical composition and property of HTCPL may be different (Saetea & Tippayawong, 2013; Funke, 2015; Idowu et al., 2017). Studies showed that HTCPL is rich in nitrogen and potassium, amino acids and other organic acids (Sun et al., 2014; Yao et al., 2016). It is noted that the major nitrogen component of HTCPL is organic nitrogen. The ammonia content of HTCPL usually is low due to the sorption of ammonia on the hydrochar (Sun et al., 2014; Funke, 2015). Sun et al. (2014) suggested that during the HTC process, a variety of organic and inorganic nutrients, such as organic acid, organic nitrogen, potassium, calcium, are produced and stored in HTCPL, which promote plant growth. Another study reported that HTCPL stimulates the protein synthesis of plants (Yao et al., 2016). However, until now there are few studies explicitly that evaluated the effect of HTCPL on plant growth and mineral composition.

### 2.1.2 Factors influencing the property and composition of HTCPL

The feedstock, heating rate, residence time, heating time, reacting temperature, reacting pressure, and particle size can jointly or independently affect the composition and property of HTCPL (Sun et al., 2014; Funke, 2015) with the feedstock and reacting temperature being the major influencing factors. HTCPL extracted from rosemary (*Rosmarinus officinalis*) may have superior antioxidant activity to other HTCPL because it has two major components i.e., IS-$\alpha$-pinene and eucalyptol during certain HTC conditions (Ma et al., 2013). In terms of major nutrients, a higher reacting temperature increases nitrogen and potassium solubilization, but the amount of dissolved phosphorus is always at a very low level (Biller et al., 2012; Saetea & Tippayawong, 2013; Sun et al., 2014; Selvaratnam et al, 2015). A previous study showed that when the reacting temperature does not exceed 200 ºC, the pH value decreases as the temperature increases, but the pattern changes when the temperature exceeds 200 ºC (Sun et al., 2014). The decrease in pH of HTCPL is due to an increase in the content of organic acids, which have the potential to promote plant growth (Sun et al., 2014).

## 2.2 The potential effects of HTCPL on plants

The results achieved from previous studies have proved the main nutrient in HTCPL is nitrogen, and the main form (over 50%) of nitrogen is organic nitrogen (Saetea & Tippayawong, 2013; Sun et al., 2014). Therefore, HTCPL may be used as organic nitrogen fertilizer. In general, the nitrogen recovered in HTCPL is positively correlated with the nitrogen content of the feedstock, reacting temperature, and/or heating time (Biller et al., 2012; Selvaratnam et al., 2015). In order to test whether the nitrogen content in HTCPL can maintain the growth of algae (*Arthrospira platensis*), Yao et al. (2016) cultured microalgae with HTCPL and compared it with sodium nitrate ($NaNO_3$), and both met the requirement of total nitrogen concentration in the culture medium. In Yao et al. (2016) experiment, HTCPL was obtained by heating algal residue at 200 °C for 3 hrs. The results showed that compared with $NaNO_3$ medium, the accumulation of carbohydrates in HTCPL medium was enhanced. This can be attributed to the sufficient photosynthetic activity maintained by the assimilation of limited organic nitrogen (Yao et al., 2016). They also found under nitrogen-limited conditions that HTCPL can be used as nitrogen source for algae, and thereby alleviating the adverse effects of nitrogen deficiency on the growth of algae. However, both the nitrogen in $NH_4^+$ and organic nitrogen in the HTCPL medium can be utilized by *A. platensis*, while nitrogen in $NO_3^-$ can hardly be used in the whole culture process.

Sun et al. (2014) used sewage sludge as feedstock for HTC, extracted HTCPL at three temperatures (180 ºC, 200 ºC and 220 ºC), and diluted the extracted solution to cultivate komatsuna (*Brassica rapa var. perviridis*). The experimental results showed that pure HTCPL may inhibit plant growth because it contains high concentrations of total organic carbon (TOC) and electrical conductivity (EC), but when diluted to an appropriate concentration, the ability of HTCPL to promote komatsuna growth was similar to traditional chemical fertilizers, especially the HTCPL obtained at 200 ºC (Sun et al., 2014). Although the solubilization of nitrogen and potassium in HTCPL can increase with the increase of reacting temperature, the contents of nitrogen and potassium in the three HTCPLs were not significantly different (Saetea & Tippayawong, 2013; Sun et al., 2014). However, the content of organic acids in HTCPL varies significantly at different heating temperatures. When the heating temperature of HTC is 200 ºC, the content of organic acids in HTCPL was the highest, and these organic acids can be used as plant growth promoters (Sun et al., 2014). Moreover, Saetea and Tippayawong (2013) used sewage sludge as HTC feedstock for investigating the value of HTC products. In Saetea and Tippayawong (2013) experiment, HTCPL was obtained by heating sewage sludge at 200 ℃ for 1, 2, 4, and 6 hours, respectively. Saetea and Tippayawong (2013) agreed with other studies that the major nutrient of HTCPL is nitrogen, but it pointed out that the heating time of HTC has no obvious effect on the chemical composition and property of HTCPL. In short, it is obvious that in the HTC process, the influence of heating temperature on the composition and property of HTCPL is clearer than heating time. Further study requires for confirming how heating time of HTC affects the chemical composition and property of HTCPL.

Although some studies suggested that HTCPL can replace traditional chemical fertilizers, other studies did not fully support this view (Sun et al., 2014; Idowu et al., 2017). In the HTC process, the fate of various chemical elements in raw materials is still unclear (Escala et al., 2012; Funke et al., 2013; Reza et al., 2013; Saetea & Tippayawong, 2013; Funke, 2015; Heilmannn et al. 2010). Taking phosphorus as an example, some studies pointed out that phosphorus in raw materials mainly exists in hydrochar (Escala et al., 2012; Heidari et al., 2018), while others suggested that phosphorus is mainly recovered in HTCPL (Heilmannn et al. 2011; Reza et al., 2013; Saetea & Tippayawong, 2013). Funke (2015) used straw digestate, corn silage and cow manure (CS-CM) digestate as raw materials and tested the fate of plant-available nutrients during the HTC process at 220 ºC heating temperature and 185 min heating time. The results showed that nitrogen and phosphorus were mainly recovered in hydrochar, while potassium was mainly recovered in HTCPL. Nitrogen was mainly found in hydrochar, probably because a large amount of nitrogen existed in ammonia form, and hydrochar has the ability to absorb ammonia (Funke, 2015). In addition, another study used three heating temperatures and five heating times as experimental variables to explore the reuse and recovery of food waste after HTC (Idowu et al., 2017). The result of this study showed that as the heating temperature and heating time increased, the specific gravity of the solid product increased. Moreover, in this study, the majority of the nitrogen was recovered in hydro char, while the majority of the potassium was recovered in HTCPL, and the fate of phosphorus was not obvious. Therefore, Idowu et al. (2017) pointed out that from the total amount of nutrients, the combination of hydro char and HTCPL can be expected to become an environmentally friendly fertilizer, but they did not suggest that HTCPL from food waste can be used alone as fertilizer.

In summary, it is not clear what the main nutrients of HTCPL are and whether it can be used as fertilizer. Due to the high correlation between the chemical elements in HTCPL and the feedstock and heating condition of HTC, it cannot be ruled out that HTCPL can recover most nutrients in raw materials under appropriate HTC conditions.

## 2.3 Brief introduction of baby greens

Baby greens require light and their growth cycle is usually 20 to 40 days (Di Gioia et al., 2017). When baby greens are harvested, they have developed more than one set of true leaves (Di Gioia et al., 2017). Baby greens have the advantages of superior flavor, rich color, various shapes and texture (Grahn et al., 2015; Baselice et al., 2017; Di Bella et al., 2020). Most of the baby greens are from Brassicaceae, Amarillydaceae, Astraceae, Lamiaceae, Apiaceae, Amaranthceae families (Paśko et al., 2009), but more and more baby greens belonging to other families are becoming popular in the market, for example, pea baby greens (Santos et al., 2014). As food, baby greens have high nutritional value with macroelements and micronutrients, and non-nutrient bioactive compounds such as protein, ascorbic acid (vitamin C), tocopherols (vitamin E), phylloquinone (vitamin K), and fiber (Francis et al. 2012; Lenzi et al., 2019; Di Bella et al., 2020). Due to the differences in crop seed size, seed weight, germination rate and expected population density, the optimum sowing rate is different for different seeds (Renna, 2016). Usually, in terms of big-seeded species such as chickpea (*Cicer arietinum*), peas (*Pisum sativum*), and sunflower, the optimal sowing rate is 1 seed / $cm^2$; in terms of smaller seeded species such as pac choi, watercress (*Nasturtium officinale*), and mustard (*Brassica juncea*), the optimal sowing rate is up to 4 seed / $cm^2$ (Di Gioia et al., 2017). Cultivating baby greens requires light and fertilizer (Di Gioia et al., 2017). It is suggested that the nutritional value and yield of baby green will be affected by the proportion and composition of fertilizer applied (Amanda et al., 2009; Aires et al., 2013; Di Mola et al., 2019). For example, a previous study has suggested that spraying lettuce (*Lactuca sativa*) baby greens with a biostimulant of an optimal concentration (Actiwave®, Valagro S.p.a) can effectively increase the chlorophyll and carotenoids content (Amanda et al., 2009). Aires et al. (2013) used conventional fertilizer and organic fertilizer to grow lettuce (*Lactuca sativa*) and watercress (*Nasturtium officinale*) baby greens separately. Their result showed that the nitrate content in baby greens depends on the type of fertilizer, and the nitrate content of baby greens cultivated with organic fertilizers was lower (Aires et al., 2013). After germination, the irrigation method is changed to sub-irrigation, which aims to prevent excessive water in the canopy of the plant and to improve product quality (Treadwell et al., 2010; Kyriacou et al., 2016).

# Chapter 3 Extraction and Comparative Analysis of Liquids Produced from Different Biomasses by Hydrothermal Process

## 3.1 Abstract

The objective of this work is to compare chemical profiles of HTCPL derived from willow (*Salix babylonica*) branches, seafood compost, and buckwheat (Fagopyrum esculentum) green foliage at different thermochemical temperatures (180 ℃ and 200 ℃) and evaluate their effects on plant growth and development. A hydrothermal treatment was performed and proved that the main contents of HTCPL are macronutrients beneficial to plants. Meanwhile, HTCPL contains trace metal substances such as copper, zinc and nickel, which are trace elements required by plants. Considering the factors of pH, hardness, electric conductivity and alkalinity of the HTCPL samples, it is recommended that the HTCPL applied to plants should be diluted. When the feedstock was the same, the contents of nitrogen, phosphorus, and magnesium were higher at 200 ℃ heating temperature. Thus, it is suggested that HTCPL from different biomass when heated at 200 ℃ has the potential to function as a bio-fertilizer.

*Key words: Hydrothermal Carbonization Processed Liquid (HTCPL), bio-fertilizer, mineral elements*

## 3.2 Introduction

Hydrothermal Carbonization Processed Liquid (HTCPL) is by-product of the hydrothermal carbonization (HTC) process, which has the potential to promote plant growth. The common heating temperature of HTC is between 180 ℃ and 350 ℃ (Saetea & Tippayawong, 2013). The feedstock to obtain HTCPL can be a variety of organic biomasses such as plant biomass, compost, and even sewage sludge (Sun et al., 2014; Funke, 2015; Yao et al., 2016; Idowu et al., 2017). There are many factors that affect the composition and property of HTCPL such as heating rate, residence time, and particle size in the HTC process (Saetea & Tippayawong, 2013; Sun et al., 2014; Funke, 2015). According to previous studies, raw materials and heating temperature are the most important factors affecting the composition and property of HTCPL (Biller et al., 2012; Saetea & Tippayawong, 2013; Sun et al., 2014; Funke, 2015).

HTCPL extracted from rosemary (*Rosmarinus officinalis*) may have superior antioxidant activity to other HTCPL because it has two major components i.e., IS-$\alpha$-pinene and eucalyptol during certain HTC conditions (Ma et al., 2013). Some studies have suggested that the nitrogen and potassium content of HTCPL increase with heating temperature (Biller et al., 2012; Saetea & Tippayawong, 2013; Sun et al., 2014; Selvaratnam et al, 2015). Moreover, the pH value of HTCPL is significantly different with changing heating temperature (Sun et al., 2014). When reacting temperature does not exceed 200 ºC, the pH value of HTCPL decreases as the temperature increases, but when the temperature exceeds 200 ºC, pH value of HTCPL decreases as the temperature decreases (Sun et al., 2014).

The feedstock and heating temperature are the major influencing factors that affect the properties and composition of HTCPL. However, the effect of feedstock and heating temperature on the fate of various mineral elements in raw materials in the HTC process is still not clear (Heilmannn et al. 2010; Escala et al., 2012; Funke et al., 2013; Reza et al., 2013; Saetea & Tippayawong, 2013; Funke, 2015). Understanding the influence of feedstock and heating temperature on the property and composition of HTCPL will be beneficial to the practical application of HTCPL in the future. The aim of this study is to compare the chemical profiles of HTCPL derived from willow (*Salix babylonica*) branches, seafood compost, and buckwheat (*Fagopyrum esculentum*) green foliage at different thermochemical temperatures (180 ℃ and 200 ℃) and to evaluate the effect of HTCPL on plant growth and development.

## 3.3 Materials and Methods

### 3.3.1 Location and materials

HTCPL extraction was performed in the Faculty of Sustainable Design Engineering, University of Prince Edward Island (UPEI) (46.15°N, 63.08°W) from May 2019 to August

2019. A pressure reactor (Parr, Moline, USA) was used to obtain the HTCPL. Seafood compost (Greenhouse Gold$^{TM}$, NB, Canada) was purchased from PEI retailers, and willow branches and buckwheat green foliage was obtained from the experimental fields of UPEI and Dalhousie University demonstration garden, Truro, respectively. The compositional analysis of HTCPL was conducted in the PEI Analytical Laboratories (Charlottetown, PEI, Canada) in August 2020.

### 3.3.2 HTCPL preparation

In each reaction, 50 g of feedstock (ground willow branches, seafood compost or buckwheat green foliage) was mixed with 450 g of distilled water in the pressure reactor without any pretreatment. After closing the reactor, the air in the reactor was purged by nitrogen to prevent oxidation during the reaction period. In the reactor, the initial pressure was set to 100 psi. After heating the pressure were be the saturated pressure at given temperature, which was from 230 psi to 260 psi. The reactor was heated to the targets of 180 ºC or 200 ºC with an average heating rate of 4.9 ºC/min. When the target temperature was reached, the mixture was kept (residence time) in the reactor for 60 min. After the heating process was completed, the reactor was cooled to room temperature (approximately 25 ºC) and the mixed solid product (hydro coal) and liquid product (HTCPL) were separated by filter paper.

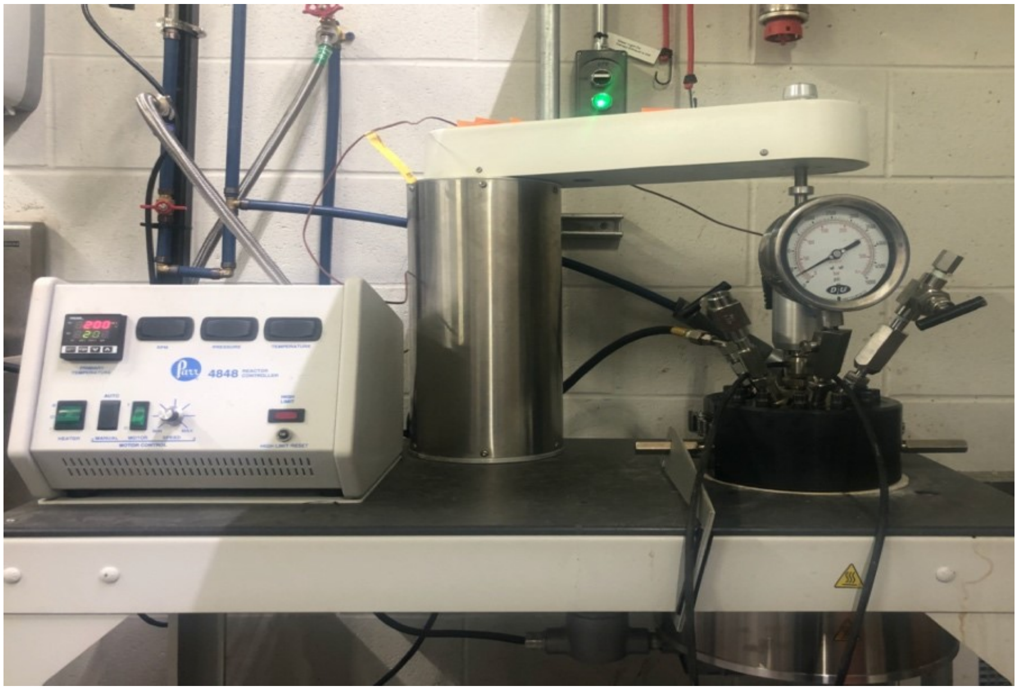



Figure 3.1 The Par 4848 reactor.

### 3.3.3 Compositional analysis

The technical support of sample compositional analysis was provided by the PEI Analytical Laboratories (Charlottetown, PEI, Canada)

*Nitrogen*

- Willow branches, seafood compost and buckwheat green foliage: By Combustion Analysis. Weighed and analyzed dried and ground samples on a CN-2000 combustion analyzer (LECO Corporation, St. Joseph, USA). Measured the content of nitrogen in willow branches, seafood compost and buckwheat green foliage by thermal conductivity detection after releasing nitrogen by combustion at high temperature.

- HTCPL samples: By colorimetric flow injection analysis.

*Other mineral elements*

- Willow branches, seafood compost and buckwheat green foliage: Ignited dried ground samples in a furnace at 550ºC to oxidize all of the organic matter. Dissolved the remaining ash in hydrochloric acid and heated. The extracts were analyzed on an Inductively Coupled Argon Plasma Optical Emission Spectrometer (5800 ICP-OES, Agilent Technologies, Santa Clara, USA).
- HTCPL samples: Solutions were analyzed on an Inductively Coupled Argon Plasma Optical Emission Spectrometer (5800 ICP-OES, Agilent Technologies, Santa Clara, USA).

*pH, Conductivity and Hardness*

- pH, Conductivity and Hardness of HTCPL samples were measured by multi-parameter water quality meter (Oakion Instruments, Illinois, USA)

### 3.3.4 Statistics analysis

Microsoft Excel was used to plot graphs. The variance-covariance structure of a set of variables were explained by the principal component analysis (PCA) using XLSTATS premium version (Addinsoft Inc, Paris, France).

## 3.4 Results

After the HTC process, the fates of some chemical elements, in willow branches, seafood compost, and buckwheat green foliage were different. For example, buckwheat HTCPLs retained most of the nitrogen in buckwheat green foliage, while the percentage of nitrogen in willow HTCPLs and SC HTCPLs was lower than the feedstocks. In addition, this work found that the composition and properties of HTCPL were more affected by feedstocks than by the heating temperature of HTC.

Table 3.1 Chemical elements in HTCPLs (hydrothermal carbonization processed liquids) obtained from SC (seafood compost), willow (*Salix babylonica*) branches, and buckwheat (*Fagopyrum esculentum*) green foliage

| **Elements (mg/kg)** | **SC (Feedstock)** | **SC HTCPL (180ºC)** | **SC HTCPL (200ºC)** |
|---|---|---|---|
| **Nitrogen** | 7500.00 | 443.00 | 486.00 |
| **Phosphorus** | 2610.00 | 1.05 | 3.50 |
| **Potassium** | 6520.00 | 3.40 | 12.85 |
| **Calcium** | 10750.00 | 84.05 | 199.60 |
| **Magnesium** | 1800.00 | 9.25 | 24.00 |
| **Boron** | 13.16 | 0.20 | 0.25 |
| **Copper** | 8.54 | 0.15 | 0.05 |
| **Zinc** | 30.60 | 0.15 | 0.30 |
| **Elements (mg/kg)** | **Buckwheat (Feedstock)** | **Buckwheat HTCPL (180ºC)** | **Buckwheat HTCPL (200ºC)** |
| **Nitrogen** | 28900.00 | 967.00 | 1467.00 |
| **Phosphorus** | 5100.00 | 207.80 | 214.20 |
| **Potassium** | 28800.00 | 1603.50 | 1574.50 |
| **Calcium** | 11400.00 | 6.20 | 6.30 |
| **Magnesium** | 6700.00 | 191.75 | 223.50 |

| **Elements (mg/kg)** | **Buckwheat (Feedstock)** | **Buckwheat HTCPL (180ºC)** | **Buckwheat HTCPL (200ºC)** |
|---|---|---|---|
| **Boron** | 20.20 | 0.85 | 0.70 |
| **Copper** | 7.40 | 0.05 | 0.05 |
| **Zinc** | 54.20 | 0.45 | 0.20 |
| **Elements (mg/kg)** | **Willow (Feedstock)** | **Willow HTCPL (180ºC)** | **Willow HTCPL (200ºC)** |
| **Nitrogen** | 2600.00 | 150.00 | 163.00 |
| **Phosphorus** | *ND | 61.95 | 68.60 |
| **Potassium** | 400.00 | 149.90 | 231.25 |
| **Calcium** | 700.00 | 131.95 | 153.85 |
| **Magnesium** | 200.00 | 44.75 | 51.75 |
| **Boron** | 2.47 | 0.70 | 0.76 |
| **Copper** | 1.57 | 0.05 | 0.05 |
| **Zinc** | 13.90 | 5.45 | 6.15 |

**ND, not detected*

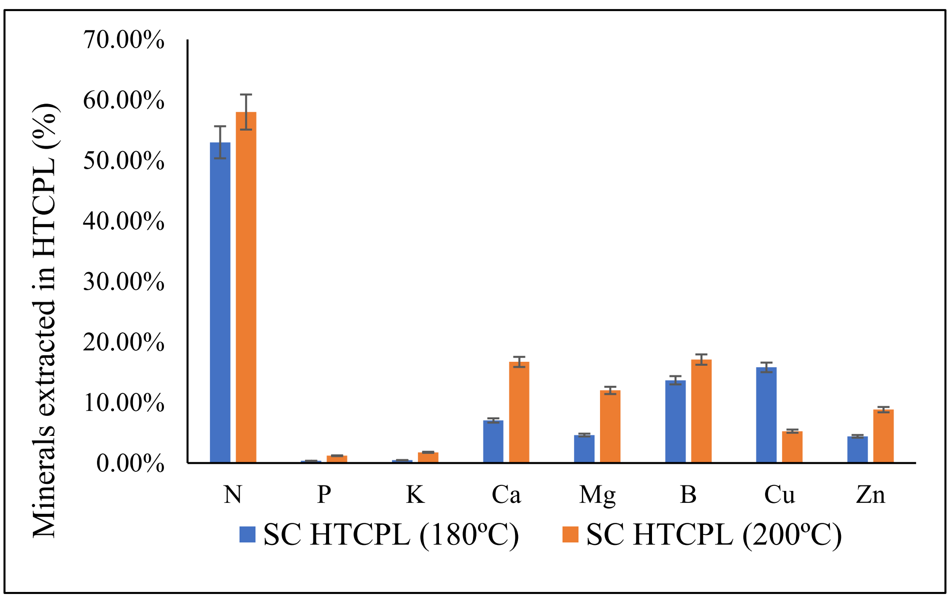



Figure 3.2 The percentage of chemical elements measured of SC (seafood compost) HTCPLs (hydrothermal carbonization processed liquids) in SC biomass.

Obviously, SC (seafood compost) HTCPLs stored most content of nitrogen of feedstock (Figure 3.2). Besides nitrogen, SC HTCPL did not retain most of mineral elements measured in the SC biomass (Figure 3.2). Comparatively, the contents of calcium, phosphorus, potassium, magnesium, boron and zinc were significantly higher in the HTCPL at 200 °C than HTCPL obtained at 180 °C (Figure 3.2).

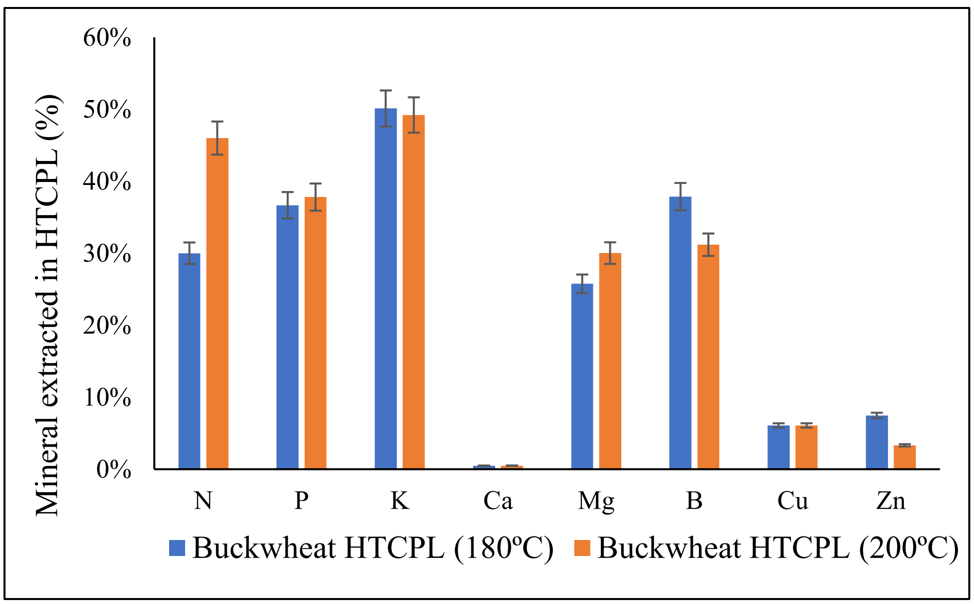



Figure 3.3 The percentage of chemical elements measured of buckwheat HTCPLs (hydrothermal carbonization processed liquids) in buckwheat (*Fagopyrum esculentum*) green foliage.

In terms of buckwheat, buckwheat HTCPLs retains half of the potassium in the feedstock. Expect for potassium, other elements measured were mainly stored in the hydro char (Figure 3.3). The buckwheat HTCPL obtained at 200 ºC had higher contents of nitrogen, calcium, magnesium and phosphorus than the buckwheat HTCPL extracted at 180 ºC, while the content of potassium in the buckwheat HTCPL at 180 °C was higher (Figure 3.3). Compared with the feedstock, the ratio of copper and zinc in buckwheat HTCPLs were low (Figure 3.3).

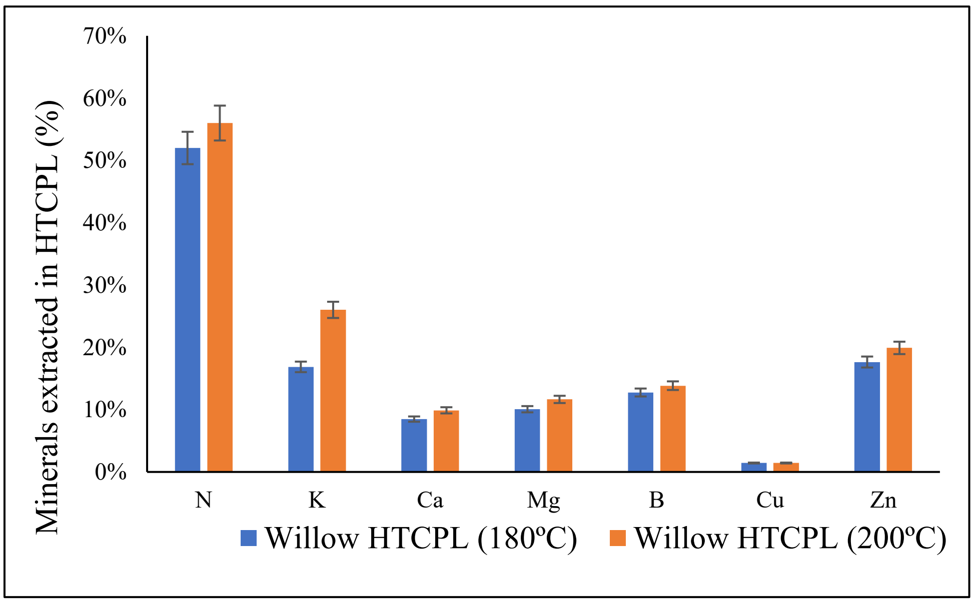
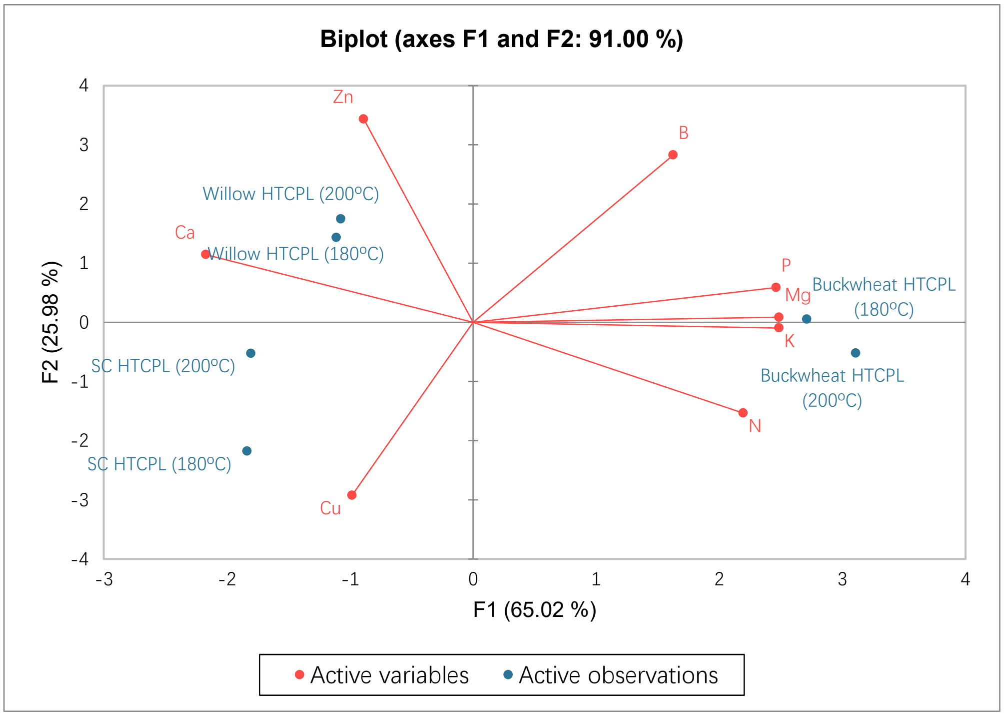



Figure 3.4 The percentage of chemical elements measured of willow HTCPLs (hydrothermal carbonization processed liquids) in willow *(Salix babylonica)* branches.

The nitrogen of willow branches was most stored in the willow HTCPLs (Figure 3.4). The willow HTCPLs did not retain the most content of copper in the feedstock (Figure 3.4). The ratio of nitrogen, potassium, calcium, magnesium boron and zinc in willow HTCPL obtained at 200 ºC was higher than that of willow HTCPL obtained at 180 ºC (Figure 3.4).




**SC, seafood compost*
**Buckwheat, buckwheat green foliage*
**Willow, willow branches*
**HTCPL, hydrothermal carbonization processed liquid*

Figure 3.5 The PCA map showed the effect of feedstock and heating temperature on the content of nitrogen, phosphorus, potassium, calcium, magnesium, boron, copper, and zinc in HTCPLs.

This segregation accounted for 91.00% of the total variability observed in the data. Based on the different feedstocks and heating temperatures, the HTCPLs were segregated into distinct quadrants on the two-dimensional principal component biplot (Figure 3.5). The output from the PCA map clearly indicated that compare with the heating temperature, the feedstock had a stronger influence on the compositions and properties of HTCPLs (Figure 3.5). And it seems that the effect of heating temperatures during HTC process on compositions and properties of HTCPLs were affected by feedstock. It was observed that the similarity of willow HTCPLs extracted at 180 ºC and 200 ºC was higher than that of SC and buckwheat HTCPLs extracted at these two temperatures (Figure 3.5).

Table 3.2 Chemical elements in HTCPLs (hydrothermal carbonization processed liquids) obtained from SC (seafood compost), willow (*Salix babylonica*) branches, and buckwheat (*Fagopyrum esculentum*) green foliage at 180 ºC and 200 ºC.

| **Elements (mg/kg)** | **SC HTCPL (180 ºC)** | **SC HTCPL (200 ºC)** | **Buckwheat HTCPL (180 ºC)** | **Buckwheat HTCPL (200 ºC)** | **Willow HTCPL (180 ºC)** | **Willow HTCPL (200 ºC)** |
|---|---|---|---|---|---|---|
| **Phosphorus** | 1.05 | 3.50 | 207.80 | 214.20 | 61.95 | 68.60 |
| **Potassium** | 3.40 | 12.85 | 1603.50 | 1574.50 | 149.90 | 231.25 |
| **Nitrogen** | 443.33 | 486.67 | 966.67 | 11466.67 | 150.00 | 163.33 |
| **Calcium** | 84.05 | 199.60 | 6.20 | 6.30 | 131.95 | 153.85 |
| **Magnesium** | 9.25 | 24.00 | 191.75 | 223.5 | 44.75 | 51.75 |
| **Copper** | 0.15 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| **Zinc** | 0.15 | 0.30 | 0.45 | 0.20 | 5.45 | 6.15 |
| **Boron** | 0.20 | 0.25 | 0.85 | 0.70 | 0.70 | 0.76 |
| **Cadmium** | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| **Chloride** | 20.00 | 30.00 | 135.00 | 130.00 | 75.00 | 100.00 |
| **Chromium** | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| **Iron** | 4.80 | 16.95 | 0.40 | 0.05 | 2.65 | 5.15 |
| **Manganese** | 4.30 | 11.10 | 2.70 | 3.00 | 6.80 | 7.95 |
| **Nickel** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Lead** | 0.25 | 0.25 | 0.25 | 1.05 | 0.25 | 2.30 |
| **Sulfate** | 27.40 | 62.65 | 160.40 | 170.35 | 43.15 | 46.65 |
| **Alkalinity** | 142.30 | 351.25 | 846.10 | 961.10 | 156.00 | 97.8 |
| **Arsenic** | 0.03 | 0.07 | 0.02 | 0.02 | 0.02 | 0.02 |
| **Hardness** | 248.00 | 597.00 | 804.50 | 935.50 | 538.50 | 597.00 |

Table 3.3 The pH value and EC in HTCPLs (hydrothermal carbonization processed liquids) obtained from SC (seafood compost), willow (*Salix babylonica*) branches, and buckwheat (*Fagopyrum esculentum*) green foliage at 180 ºC and 200 ºC.

| | **SC HTCPL (180 ºC)** | **SC HTCPL (200 ºC)** | **Buckwheat HTCPL (180 ºC)** | **Buckwheat HTCPL (200 ºC)** | **Willow HTCPL (180 ºC)** | **Willow HTCPL (200 ºC)** |
|---|---|---|---|---|---|---|
| **pH** | 6.54 | 5.1 | 4.34 | 4.56 | 3.98 | 3.73 |
| **EC (μohms /cm)** | 111.2 | 296 | 1437 | 1469 | 491 | 590 |

Nitrogen, phosphorus, potassium, calcium, magnesium and sulfur are major elements required for plant growth. Some elements play an important role in plant growth but are required in small amounts by plants and are called micronutrients including chlorine, iron, boron, manganese, zinc, copper, and nickel. Analysis of mineral elements showed that the contents of phosphorus and potassium in the willow and buckwheat HTCPLs were more than 20-fold compared to that of the SC HTCPLs (Table 3.2). The contents of magnesium and sulfate of the buckwheat HTCPLs were significantly higher than that of the willow and SC HTCPLs (Table 3.2). Also, the content of electric conductivity and alkalinity of the buckwheat HTCPLs were significantly higher than that of the willow and SC HTCPLs (Table 3.3). The willow HTCPL had the lowest pH value at 3.98 for the 180 ℃ and 3.73 for the 200 ℃ extraction temperatures (Table 3.3). Hardness is positively correlated with the mineral content in the solution. It can be found that for the same feedstock, the hardness value of the HTCPL extracted with 200 ℃ was higher than the HTCPL obtained with 180 ℃ (Table 3.2).

## 3.5 Discussion

The fate of feedstock nitrogen in the HTC process is controversial (Funke, 2015; Funke et al., 2013; Idowu et al., 2017; Heilmann et al., 2010). Some studies believe that after experiencing HTC, the total nitrogen in the feedstocks is mainly stored in HTCPL, while other studies believe that the total nitrogen is mainly stored in hydrochar (Heilmann et al., 2010; Funke et al., 2013; Funke, 2015; Idowu et al., 2017). In addition, studies have also suggested that the total nitrogen in the feedstocks is stored in HTCPL and hydrochar on average (Heilmann et al., 2010; Funke et al., 2013; Funke, 2015; Idowu et al., 2017). The result of this work supports the existence of controversy over the fate of nitrogen. Obviously, willow HTCPLs and SC HTCPLs retain most of the nitrogen derived from the feedstock, while the nitrogen in buckwheat green foliage was stored in the solid product (Figure 3.2 - 3.4). It is suggested that the nitrogen in the HTC process has a higher correlation with the feedstocks than the heating temperature. Previous studies reported that heating temperature is one of the main factors that determine the fate of phosphorus in the HTC process (Idowu et al., 2017). This is because the dissolution of phosphorus is strongly related to the pH of the solution, and the pH of HTCPL is affected by the heating temperature of HTC. Generally, when the HTC reaction temperature is less than 200 ºC, the pH of HTCPL is decreased as the temperature increases (Sun et al., 2014). The results of the present study supported the above report by Sun et al. (2014). That is, the HTCPLs from the same sources indicated that the pH of liquids obtained at 200 ºC was lower than those extracted at 180 ºC (Table 3.1). As the pH increases, the precipitation of phosphate formed by phosphorus and cations increases, and the adsorption capacity of hydrochar to phosphorus was also enhanced (Idowu et al., 2017). The phosphorus content in willow was not accounted for in the present study. However, it can be speculated that phosphorus in willow feedstock was released in the willow HTCPL due to the low pH value of its HTCPLs (Table 3.1). Potassium is a cation with greater solubility in liquids than calcium and magnesium (Idowu et al., 2017). Calcium and magnesium are higher in acidic HTCPL because precipitation is not easy to occur under acidic conditions (Idowu et al., 2017). In this work, the effect of heating temperature on the contents of magnesium and calcium in HTCPLs was the most significant when the feedstock was SC (Table 3.1 & Figure 3.2). This implies HTC feedstocks and heating time influence the fate of minerals. Boron, copper and zinc are basically not retained in HTCPL (Table 3.1), which may also be due to precipitation and solid adsorption (Idowu et al., 2017).

Obviously, feedstocks and heating temperature affected the compositions and properties of the HTCPLs. For example, willow contains abundant acetic acid and other carboxylic acids (Hager et al., 2020) and thus, the pH of the HTCPLs extracted from willow is the least (Table 3.1). The fate of phosphorus in the HTC process is affected by heating time (Idowu et al., 2017). If the feedstocks were the same, the phosphorus content of HTCPLs extracted at 200 ºC was higher than that of HTCPLs extracted at 180 ºC (Table 3.2). The results of this work showed that HTCPLS from different biomasses rich in different kind of macronutrients for plant. For example, buckwheat HTCPL rich in nitrogen, potassium, phosphorus and magnesium, and willow HTCPL not only rich in the above elements also rich in calcium (Table 3.2). In addition, it was found that HTCPL contained trace elements required by plants, and these included chlorine, copper, zinc, boron, nickel, and iron (Table 3.2). Therefore, it suggests that HTCPL has the potential to be used in plant fertilization. Electric conductivity of solution can be seen as the measure of the solution's ability to transfer an electrical current (Sun et al., 2014). A proper electric conductivity is beneficial for the exchange of substances between plants and their surrounding environment. but when the electric conductivity of the nutrient solution exceeds 700 μohms /cm, the growth of the plant will be negatively affected (Fang & Wong, 1999; Fuentes et al., 2004; Sun et al., 2014). Therefore, it is suggested that if using the willow HTCPLs and buckwheat HTCPLs to cultivate plants, the liquids have to be diluted (Table 3.3). According to the recorded pH, willow HTCPLs and buckwheat HTCPLs should also be used for plant cultivation after dilution because the pH suitable for most plants' growth is higher than 5 (Roem et al., 2002; Koger et al., 2004; Liu & Hanlon, 2015). Alkalinity is a different concept from pH. Alkalinity of water is used to measure the carbonate and bicarbonate levels, and water pH is the measures of the amount of hydrogen (acid ions) (Roosta, 2011). However, alkalinity can directly affect the pH in liquids (Roosta, 2011). Alkalinity and pH show a positive correlation (Kuehny & Morales, 1998). High alkalinity will inhibit the ability of plants to absorb iron and thereby, reducing the synthesis of chlorophyll (De laGuardia, & Alcántara, 2002). Usually, the range of alkalinity suitable for plant growth is 0 to 160 mg/kg (Roosta, 2011). Lead, arsenic, cadmium and other elements were detected in HTCPLs, and their contents were low (Table 3.2). It is inferred that elemental pollution will not cause significant negative effects on plant growth after all HTCPLs of this study are diluted. Water hardness usually indicates the content of calcium carbonate in the solution (Veríssimo et al., 2007). For plants, hard water (120 mg/kg – 180 mg/kg) and very hard water (over 180 mg/kg) are not conducive to the exchange of materials between plants and soil, because hard water or very hard water may form a layer of calcium carbonate or other carbonates on the roots and soil (Veríssimo et al., 2007). Treating plants with diluted HTCPL samples can avoid the negative impact of solution hardness on plants. When the feedstock was the same, the contents of nitrogen, phosphorus, and magnesium were higher for the 200 ℃ heating temperature (Table 3.2). Therefore, it was suggested that HTCPL extracted at 200 ℃ has more potential for use as fertilizer than HTCPL extracted at 180 ℃. The output from the PCA map shows that raw materials have more influence on the compositions and properties of HTCPLs than heating temperature (Figure 3.5), which is the same as the conclusions of previous studies.

### 3.6 Conclusion

Overall, the study showed that the fates of mineral elements are not fixed during the HTC process and can vary. The compositions and properties of the feedstock and the heating temperature during the HTC process affect the fate of mineral elements. The HTCPLs have the macronutrients needed for plant. Also, trace elements needed for the plants contained in HTCPLs. Thus, it is inferred that HTCPL may be used for plant cultivation in the future. Due to the concentration of mineral elements and nature of HTCPL, it is recommended to test the effect of HTCPL on plants after diluting the liquids.

## Chapter 4 Seed Germination and Seedling Growth Response to Hydrothermal Carbonization Processed Liquids Extracted from Different Biomasses

### 4.1 Abstract

Hydrothermal carbonization processed liquid (HTCPL) is a by-product of hydrothermal carbonization of biomass, which can be used as a bio-fertilizer. A study was performed to evaluate the chemical composition and efficacies of HTCPL obtained from three biomass feedstock, namely, willow (*Salix babylonica*), buckwheat (*Fagopyrum esculentum*) and seafood compost (SC) by using 200 ºC extraction temperature. Pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*) and lettuce (*Lactuca sativa*) seeds germination and seedlings growth and establishment in different concentrations of the individual HTCPLs were evaluated. Both the 5% and the 10% of the willow HTCPLs showed negative effects on the germination of seeds and growth of seedlings. The SC HTCPLs, buckwheat HTCPLs with 0.5% concentrations of willow HTCPLs had no significant effect on seed germination and seedling growth. This work suggestion that HTCPL cannot significantly promote seed germination and seedling growth

*Keywords: waste product, hydrothermal carbonization, bio-fertilizer, seed germination, seedling growth*

## 4.2 Introduction

Hydrothermal Carbonization (HTC) is a thermochemical process that converts organic materials to value-added products at moderate temperatures. The common HTC reaction temperature ranges from 180 ℃ to 350 ℃ (Saetea & Tippayawong, 2013). The main outcome of HTC is hydro char. Hydrothermal Carbonization Processed Liquid (HTCPL), a by-product of HTC process, has the potential to be applied flexibly as a fertilizer (Sun et al., 2014; Funke, 2015; Yao et al., 2016). Many organic biomasses can be used as feedstock for HTC such as various plants, composts, and even sewage sludge (Saetea & Tippayawong, 2013; Funke, 2015; Yao et al., 2016; Idowu et al., 2017). Previous studies have shown that the main plant nutrient in HTCPL is nitrogen with more than 50% in its organic form; hence, HTCPL may be used as an organic nitrogen fertilizer (Saetea & Tippayawong, 2013; Sun et al., 2014). Under nitrogen-limited conditions, HTCPL extracted from algae (*Arthrospira platensis*) can be used as a nitrogen source for algae, thereby alleviating the adverse effects of nitrogen deficiency on the growth of algae (Yao et al., 2016). Depending on feedstocks, heating rate, residence time, heating temperature, and particle size during the HTC process, the chemical compositions and properties of HTCPL may be different (Saetea & Tippayawong, 2013; Funke, 2015; Idowu et al., 2017).

Although some studies suggested that HTCPL can replace traditional chemical fertilizers, other studies did not fully support this view (Sun et al., 2014; Idowu et al., 2017). In the HTC process, the fate of various chemical elements in raw materials is still unclear (Heilmannn et al. 2010; Escala et al., 2012; Funke et al., 2013; Reza et al., 2013; Saetea & Tippayawong, 2013; Funke, 2015). Taking phosphorus as an example, some studies pointed out that phosphorus in raw materials mainly exists in hydro char (Escala et al., 2012; Heidari et al., 2018), while others suggested that phosphorus is mainly recovered in HTCPL (Heilmannn et al. 2011; Reza et al., 2013; Saetea & Tippayawong, 2013). Moreover, one study found that after HTC of food waste, the majority of the nitrogen was recovered in hydro char, while the majority of the potassium was recovered in HTCPL, and the fate of phosphorus had no obvious bias (Idowu et al., 2017). Therefore, the researchers pointed out that from the total amount of nutrient, the combination of hydro char and HTCPL can be expected to become an environmental friendly fertilizer, but the study didn't suggest that HTCPL from food waste alone can be used as a fertilizer for agriculture (Idowu et al., 2017).

In summary, it is not clear what compositions of HTCPL can be used as fertilizer or bio-stimulant. However, due to the great correlation between the chemical elements in HTCPL and the feedstock and heating condition of HTC, it cannot be ruled out that HTCPL can recover most nutrients in raw materials under appropriate HTC conditions. To evaluate the chemicals and the efficacy of HTCPL extracted from different biomasses, this research project focuses on investigating the seed germination and the development of radicle (embryonic root) and hypocotyl (embryonic shoot) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) response to HTCPL derived from willow (*Salix babylonica*) branches, seafood compost, and buckwheat (*Fagopyrum esculentum*) green foliage.

## 4.3. Materials and Methods

### 4.3.1 Location and materials

The HTCPL extraction was produced in the lab of the Faculty of Sustainable Design Engineering, University of Prince Edward Island (UPEI) from May 2019 to August 2019. The experiments of seed germination, and hypocotyl and radicle development were performed at the Department of Plant, Food, and Environmental Sciences, Dalhousie University Agricultural Campus (45.37°N, 63.26°W) from September 2019 to April 2020. Seafood compost (Greenhouse Gold$^{TM}$, NB, Canada) was purchased from PEI retailers, and willow branches and buckwheat green foliage was obtained from the experimental field of UPEI and experimental field of Dalhousie Agriculture campus, respectively.

Organically certified kale, pac choi, peas, sunflower and lettuce seeds were purchased from West Coast Seed™ (BC, Canada).

4.3.2 Procedures

This work can be divided into two experiments. The practical processes between experiment 1 and experiment 2 were similar, but the treatments and objects have difference. HTCPLs used in this work were obtained from SC, buckwheat green foliage and willow branches by using 200 ºC reacting temperature. The different concentrations (%) of HTCPL (e.g., 5%) means diluted the extracted HTCPL to that concentration.

Table 4.1 Feedstocks and process conditions used to produce HTCPL (hydrothermal carbonization processed liquid) based on willow (*Salix babylonica*) branches, SC (seafood compost), and buckwheat (*Fagopyrum esculentum*) green foliage.

| **Feedstock** | **Willow branches** | **SC** | **Buckwheat green foliage** |
|---|---|---|---|
| **Process temperature ºC** | 200 | 200 | 200 |
| **Initial pressure psi** | 100 | 100 | 100 |
| **Process heating rate ºC/min** | 4.9 | 4.9 | 4.9 |
| **Process time min** | 60 | 60 | 60 |

**SC, seafood compost*

Table 4.2 Chemical elements in different concentrations of HTCPLs diluted (hydrothermal carbonization processed liquids) extract from SC (seafood compost), buckwheat (*Fagopyrum esculentum*) green foliage and willow (*Salix babylonica*) branches.

| Element (mg/kg) | 5% SC HTCPL | 10% SC HTCPL | 0.5% Buckwheat HTCPL | 1% Buckwheat HTCPL | 2.5% Buckwheat HTCPL | 5% Buckwheat HTCPL |
|---|---|---|---|---|---|---|
| **Phosphorus** | 0.18 | 0.35 | 1.07 | 2.14 | 5.36 | 10.71 |
| **Nitrogen** | 24.33 | 48.67 | 7.33 | 14.67 | 36.67 | 73.33 |
| **Potassium** | 0.64 | 1.29 | 7.87 | 15.75 | 39.36 | 78.73 |
| **Calcium** | 9.98 | 19.96 | 0.03 | 0.06 | 0.16 | 0.32 |
| **Magnesium** | 1.20 | 2.40 | 1.12 | 2.24 | 5.59 | 11.18 |
| **Copper** | <0.0025 | <0.005 | 0.00025 | 0.0005 | 0.00125 | 0.0025 |
| **Zinc** | 0.02 | 0.03 | 0.001 | 0.002 | 0.005 | 0.01 |
| **Boron** | 0.0125 | 0.03 | 0.0035 | 0.007 | 0.0175 | 0.035 |
| **Cadmium** | <0.005 | <0.01 | <0.0005 | <0.001 | <0.0025 | <0.005 |
| **Chloride** | 1.50 | 3.00 | 0.65 | 1.30 | 3.25 | 6.50 |
| **Chromium** | <0.005 | <0.01 | <0.0005 | <0.001 | <0.0025 | <0.005 |
| **Iron** | 0.85 | 1.70 | 0.00025 | 0.0005 | 0.00125 | 0.0025 |
| **Manganese** | 0.56 | 1.11 | 0.02 | 0.03 | 0.08 | 0.15 |
| **Nickel** | <0.025 | <0.05 | <0.0025 | <0.005 | <0.0175 | <0.025 |
| **Lead** | <0.00125 | <0.03 | 0.00525 | 0.0105 | 0.02625 | 0.0525 |
| **Sulfate** | 3.13 | 6.27 | 0.85 | 1.70 | 4.26 | 8.52 |
| **Alkalinity** | 17.56 | 35.13 | 4.81 | 9.61 | 24.03 | 48.06 |
| **Arsenic** | 0.0035 | 0.01 | <0.0001 | <0.0002 | <0.0005 | <0.001 |
| **Hardness** | 29.85 | 59.70 | 4.68 | 9.36 | 23.39 | 46.78 |

| Element (mg/kg) | 10% Buckwheat HTCPL | 0.5% Willow HTCPL | 1% Willow HTCPL | 2.5% Willow HTCPL | 5% Willow HTCPL | 10% Willow HTCPL |
|---|---|---|---|---|---|---|
| **Phosphorus** | 21.42 | 0.34 | 0.69 | 1.72 | 3.43 | 6.86 |
| **Nitrogen** | 146.67 | 0.82 | 1.63 | 4.08 | 8.17 | 16.33 |
| **Potassium** | 157.45 | 1.16 | 2.31 | 5.78 | 11.56 | 23.13 |
| **Calcium** | 0.63 | 0.77 | 1.54 | 3.85 | 7.69 | 15.39 |
| **Magnesium** | 22.35 | 0.26 | 0.52 | 1.29 | 2.59 | 5.18 |
| **Copper** | 0.005 | 0.00025 | 0.0005 | 0.00125 | 0.0025 | 0.005 |
| **Zinc** | 0.02 | 0.03 | 0.06 | 0.15 | 0.31 | 0.62 |
| **Boron** | 0.07 | 0.00325 | 0.0065 | 0.01625 | 0.0325 | 0.065 |
| **Cadmium** | <0.01 | <0.0005 | <0.001 | <0.0025 | <0.005 | <0.01 |
| **Chloride** | 13.00 | 0.50 | 1.00 | 2.50 | 5.00 | 10.00 |

| Element (mg/kg) | 10% Buckwheat HTCPL | 0.5% Willow HTCPL | 1% Willow HTCPL | 2.5% Willow HTCPL | 5% Willow HTCPL | 10% Willow HTCPL |
|---|---|---|---|---|---|---|
| **Iron** | 0.01 | 0.03 | 0.05 | 0.13 | 0.26 | 0.52 |
| **Chromium** | <0.01 | <0.0005 | <0.001 | <0.0025 | <0.005 | <0.01 |
| **Manganese** | 0.30 | 0.04 | 0.08 | 0.20 | 0.40 | 0.80 |
| **Nickel** | <0.05 | <0.0025 | <0.005 | <0.0175 | <0.025 | <0.05 |
| **Lead** | 0.11 | 0.01 | 0.02 | 0.06 | 0.12 | 0.23 |
| **Sulfate** | 17.04 | 0.23325 | 0.47 | 1.17 | 2.33 | 4.67 |
| **Alkalinity** | 96.11 | 0.49 | 0.98 | 2.45 | 4.89 | 9.78 |
| **Arsenic** | <0.002 | <0.0001 | <0.0002 | <0.0005 | <0.001 | <0.002 |
| **Hardness** | 93.55 | 2.99 | 5.97 | 14.93 | 29.85 | 59.70 |

Table 4.3 pH value of different concentrations of HTCPLs (hydrothermal carbonization processed liquids) extract from SC (seafood compost), buckwheat (*Fagopyrum esculentum*) green foliage and willow (*Salix babylonica*) branches.

| | 5% SC HTCPL | 10% SC HTCPL | 0.5% Buckwheat HTCPL | 1% Buckwheat HTCPL | 2.5% Buckwheat HTCPL | 5% Buckwheat HTCPL |
|---|---|---|---|---|---|---|
| **pH** | 5.75 | 5.21 | 5.51 | 5.37 | 5.01 | 4.66 |
| | **10% Buckwheat HTCPL** | **0.5% Willow HTCPL** | **1% Willow HTCPL** | **2.5% Willow HTCPL** | **5% Willow HTCPL** | **10% Willow HTCPL** |
| **pH** | 4.58 | 4.87 | 4.58 | 4.33 | 4.04 | 3.75 |

Experiment 1:

- Seed germination and seedling growth of kale, pac choi, peas, sunflower and lettuce response to 5% and 10% HTCPLs from different biomass

Seeds of kale, pac choi, peas, sunflower and lettuce were used as the objects, moreover, this part has two independent variables: 1) three sources of biomass for HTCPL i.e. seafood compost, willow and buckwheat; 2) two different concentrations i.e. 5% and 10% with water as a control. Depending on the two independent variables, the experiment had seven treatments: 0 (water), 5% willow HTCPL, 10% willow HTCPL, 5% seafood compost HTCPL, 10% seafood compost HTCPL, 5% buckwheat HTCPL, and 10% buckwheat HTCPL. Seeds were placed in the trough of seed germination pouches ($CYG_{TM}$, Newport, USA). Due to the different seed sizes, not all kinds of seeds have the same replications. For pea and sunflower, this experiment repeated 4 times, and each seed germination pouch holds 10 seeds. In terms of kale, lettuce and pac choi, there were 3 replications, and 20 seeds of each species were put in one seed germination pouch. Depending on the instruction of the pouches were used, 20 ml of the solutions were added to the bottom of each pouch. The 5 ml of sample solutions were added every two days to ensure pouches stay wet. When the radicle of the seed was visible, the seed was considered to be a germinated seed. The experiment ended when the number of seed germinations did not increase for three consecutive days.

Experiment 2:

- Seed germination and seedling growth of kale, response to 0.5%, 1%, and 2.5% HTCPLs from different biomass

The experimental treatments were: 1) two sources of biomass for HTCPL i.e., willow and buckwheat; 2) three different concentrations i.e., 0.5% 1%, and 2.5% with water as a control. Depending on the two independent variables, the experiment had seven treatments:

0 (water), 0.5% willow HTCPL, 1% willow HTCPL, 2.5% willow HTCPL, 0.5% buckwheat HTCPL, 1% buckwheat HTCPL, and 2.5% buckwheat HTCPL. Kale seeds were placed in the trough of seed germination pouches ($CYG_{TM}$, Newport, USA). There were three replications, and 20 seeds of each species were put in one seed germination pouch. 20 ml of the solutions were added to the bottom of each pouch. The 5 ml of sample solutions were added every two days to ensure that the seeds stay wet. The seed was considered germinated when the radicle of the seed was visible. The experiment ended when the number of seed germinations did not increase for three consecutive days.

Data Collection:

*Seed germination parameter*

To obtain the germination rate, germination potential, germination index, and seed vigor index, the sum of germinating seeds and the number of days of seeds germinated were recorded every day until the end of the experiment. The mathematical expressions were given by:

Germination rate (%) = $\frac{sum\ of\ germinating\ seeds}{the\ total\ number\ of\ seeds} \times 100$ (Yang et al., 2015).

Germination potential (%) = $\frac{sum\ of\ germinating\ seeds\ when\ germinations\ reach\ peak}{the\ total\ number\ of\ seeds} \times 100$ (Yang et al., 2015).

Germination index = $\sum(G_t \div D_t)$, where $G_t$ = the number of seeds germinated at day and $D_t$ = the corresponding days of germination (Yang et al., 2015).

Seed vigor index = Germination rate $\times Mean \frac{S_i+R_i}{100}$, where $S_i$ = the shoot length and $R_i$ = the root length (Yousefi et al., 2017).

*Seedling growth parameters*

The lengths of hypocotyls and radicles and the surface areas of hypocotyls and radicles were measured with a STD4800 scanner (Epson, Long Beach, USA) on the last day of the measurement. The digital images of seedlings were obtained by the STD4800 scanner (Epson, Long Beach, USA) as well.

4.3.3 Statistical analysis

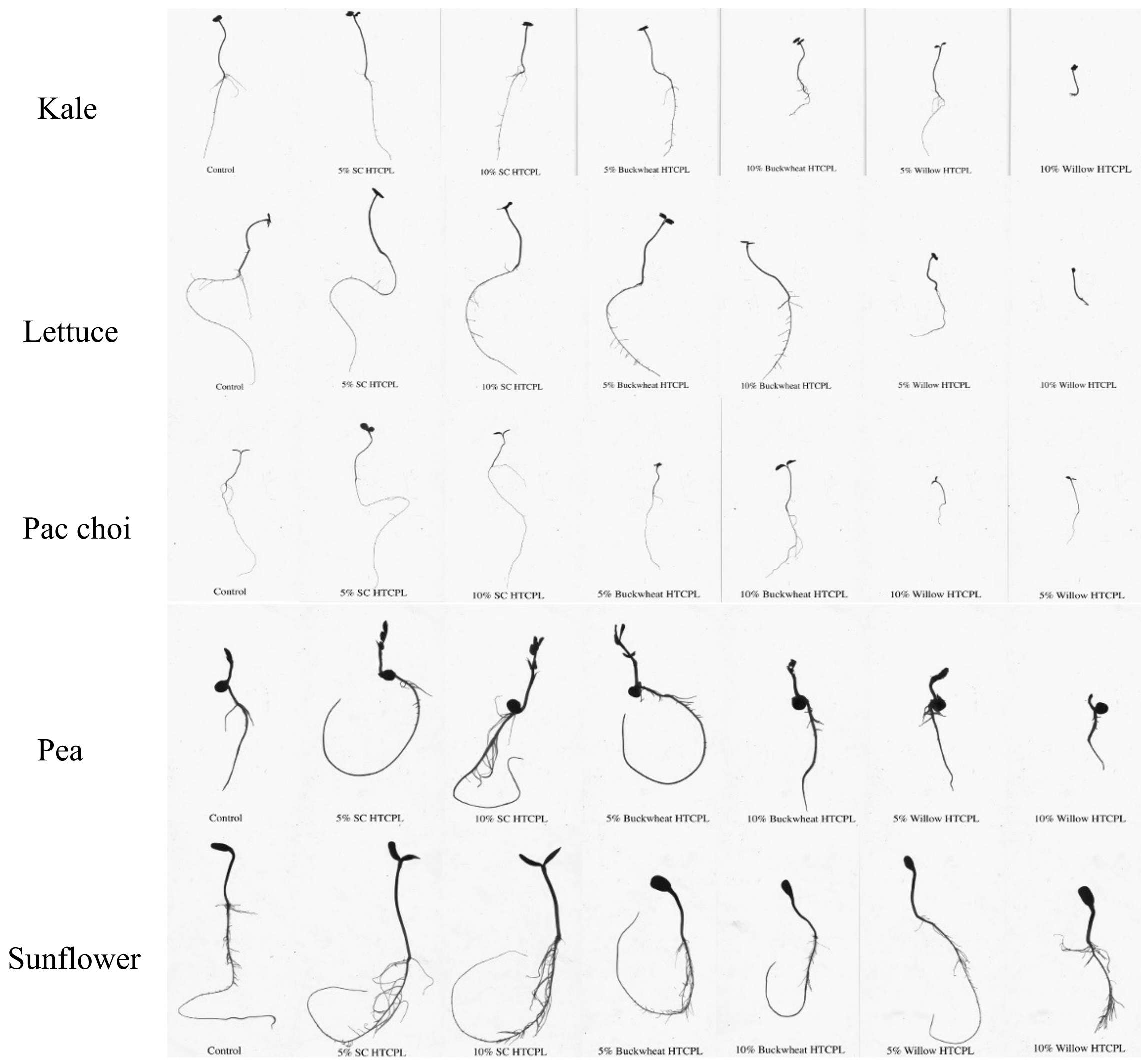
For Experiment 1, data were subjected to a three-factorial design of analyses of variance (ANOVA) using Minitab version 18.1 (Minitab Inc., State College, PA, USA). Tukey's method was used to separate treatment means when the ANOVA indicated a significant difference at $P \leq 0.05$. The variance-covariance structure of a set of variables was explained by two-dimensional principal component analysis (PCA) using Minitab version 18.1 (Minitab Inc., State College, PA, USA). Microsoft Excel was used to plot graphs. For Experiment 2, data were subjected to a two-factor factorial design of ANOVA using Minitab. Tukey's method was used to separate treatment means when the ANOVA indicated a significant difference at $P \leq 0.05$. Microsoft Excel was used to plot graphs.

## 4.4. Results

Experiment 1

- Seed germination and seedling growth of kale, pac choi, peas, sunflower and lettuce response to 5% and 10% HTCPLs from different biomass

HTCPLs did not significantly promote seed germination and seedling growth, and willow HTCPLs showed a negative effect (Figure 1).




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*

**Willow, willow (Salix babylonica) branches*

Figure 4.1 The morphology of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

Table 4.4 The average germination rate (%), germination index, germination potential (%), seed vigor index, radicle length (cm), hypocotyl length (cm), radicle surface area (cm2) and hypocotyl surface area (cm2) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

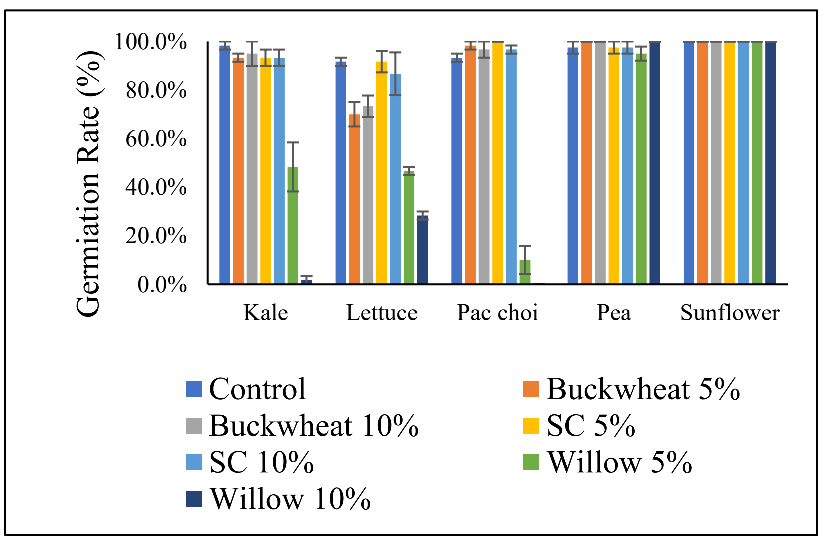
| | **Treatments** | **Germination Rate (%)** | **Germination Index** | **Germination Potential (%)** | **Seed Vigor Index** |
|---|---|---|---|---|---|
| | Control | 98.3 a | 5.76 cd | 66.7 a…g | 2.00 abc |
| | Buckwheat 5% | 93.3 a | 5.83 cd | 78.3 a…e | 1.54 a…f |
| | Buckwheat 10% | 95.0 a | 5.83 cd | 73.3 a…f | 1.37 b…g |
| **Kale** | SC 5% | 93.3 a | 5.59 cde | 63.3 a…g | 1.90 a…g |
| | SC 10% | 93.3 a | 5.90 c | 70.0 a…g | 1.53 a…f |
| | Willow 5% | 48.3 c | 1.94 ijk | 35.0 f…j | 0.40 hij |
| | Willow 10% | 1.7 e | 0.04 l | 1.7 j | 0.00 j |
| | Control | 91.7 a | 7.55 b | 60.0 b…h | 1.15 b…h |
| | Buckwheat 5% | 70.0 b | 4.27 efg | 40.0 d…j | 0.68 g…j |
| | Buckwheat 10% | 73.3 b | 4.47 def | 38.3 e…j | 0.79 f…j |
| **Lettuce** | SC 5% | 91.7 a | 6.96 bc | 48.3 c…i | 1.54 c…i |
| | SC 10% | 86.7 ab | 5.81 cd | 30.0 g…j | 1.11 d…h |
| | Willow 5% | 46.7 c | 2.40 hij | 30.0 g…j | 0.32 hij |
| | Willow 10% | 28.3 d | 1.08 jkl | 21.7 hij | 0.26 ij |
| | Control | 93.3 a | 7.78 ab | 65.0 a…g | 1.03 a…g |
| | Buckwheat 5% | 98.3 a | 9.08 a | 76.7 a…e | 1.08 d…i |
| **Pac choi** | Buckwheat 10% | 96.7 a | 7.91 ab | 81.7 abc | 0.64 g…j |
| | SC 5% | 100.0 a | 8.21 ab | 85.0 abc | 1.01 e…i |

| | | | | | |
|---|---|---|---|---|---|
| **Pac choi** | SC 10% | 96.7 a | 7.97 ab | 83.3 abc | 1.05 e…i |
| | Willow 5% | 10.0 e | 0.83 kl | 10.0 ij | 0.04 j |
| | Willow 10% | 0.0 e | 0.06 l | 1.7 j | 0.00 j |
| **Pea** | Control | 97.5 a | 2.75 hi | 85.0 ab | 0.94 f…i |
| | Buckwheat 5% | 100.0 a | 3.15 f…i | 80.0 abc | 1.52 a…f |
| | Buckwheat 10% | 100.0 a | 2.80 hi | 87.5 ab | 1.00 e…i |
| | SC 5% | 97.5 a | 3.02 ghi | 70.0 a…f | 1.37 b…g |
| | SC 10% | 97.5 a | 3.06 ghi | 77.5 a…d | 1.42 b…g |
| | Willow 5% | 95.0 a | 2.99 ghi | 75.0 a…e | 0.88 f…i |
| | Willow 10% | 100.0 a | 2.95 ghi | 82.5 abc | 0.48 hij |
| **Sunflower** | Control | 100.0 a | 3.29 fgh | 95.0 ab | 1.5 a…f |
| | Buckwheat 5% | 100.0 a | 3.33 fgh | 100.0 a | 1.71 a…e |
| | Buckwheat 10% | 100.0 a | 3.29 fgh | 95.0 ab | 1.33 c…g |
| | SC 5% | 100.0 a | 3.29 fgh | 95.0 ab | 2.09 ab |
| | SC 10% | 100.0 a | 3.33 fgh | 100.0 a | 2.20 a |
| | Willow 5% | 100.0 a | 3.33 fgh | 100.0 a | 1.55 a…f |
| | Willow 10% | 100.0 a | 3.33 fgh | 100.0 a | 1.08 e…h |

| | **Treatments** | **Radicle Length (cm)** | **Hypocotyl Length (cm)** | **Radicle Surface area ($cm^2$)** | **Hypocotyl Surface area ($cm^2$)** |
|---|---|---|---|---|---|
| **Kale** | Control | 47.4 abc | 20.6 abc | 8.0 a…f | 4.4 def |
| | Buckwheat 5% | 38.1 a…g | 17.2 b…e | 7.2 a…g | 4.1 e…h |
| | Buckwheat 10% | 30.4 d…i | 17.1 b…e | 6.3 c…i | 3.9 fgh |
| | SC 5% | 49.0 ab | 19.2 bcd | 8.1 a…e | 3.5 f…i |
| | SC 10% | 39.5 a…f | 15.2 b…f | 7.5 a…g | 7.5 ab |
| | Willow 5% | 18.2 i…m | 7.9 f…l | 4.6 h…k | 2.2 jkl |
| | Willow 10% | 0.7 n | 0.5 l | 0.2 m | 0.2 m |
| **Lettuce** | Control | 29.1 e…j | 11.6 d…j | 6.1 d…j | 3.2 hi |
| | Buckwheat 5% | 20.9 h…m | 7.4 f…l | 5.1 e…j | 2.7 ijk |
| | Buckwheat 10% | 20.5 h…m | 11.6 d…j | 5.6 f…j | 3.4 ghi |
| | SC 5% | 46.6 abc | 13.7 b…g | 8.5 a…d | 2.9 h…k |
| | SC 10% | 33.8 b…i | 10.7 e…k | 6.9 a…i | 6.9 bc |
| | Willow 5% | 7.8 lmn | 6.9 g…l | 3.1 kj | 2.7 ijk |
| | Willow 10% | 5.1 mn | 4.5 jkl | 2.0 lm | 2.0 jk |
| **Pac choi** | Control | 23.2 g…l | 13.7 b…g | 5.9 e…j | 3.4 ghi |
| | Buckwheat 5% | 23.1 g…l | 13.3 b…g | 5.2 g…k | 3.5 f…i |
| | Buckwheat 10% | 11.9 k…n | 10.0 e…k | 3.7 jkl | 3.6 f…i |
| | SC 5% | 21.1 h…m | 12.4 d…i | 5.2 g…k | 3.6 f…i |

| | | | | | |
|---|---|---|---|---|---|
| **Pac choi** | SC 10% | 22.9 g…l | 13.1 b…h | 5.9 e…j | 5.9 c |
| | Willow 5% | 5.2 mn | 9.0 f…k | 1.8 lm | 1.9 jk |
| | Willow 10% | 0.3 n | 0.4 l | 0.1 m | 0.2 m |
| **Pea** | Control | 27.5 f…j | 4.2 kl | 6.9 a…i | 2.6 ijk |
| | Buckwheat 5% | 41.5 a…e | 9.2 f…k | 8.6 ab | 4.1 e…h |
| | Buckwheat 10% | 28.0 e…j | 5.4 i…l | 7.9 a…e | 3.9 fgh |
| | SC 5% | 39.2 a…f | 7.6 g…l | 8.4 abc | 5.1 cde |
| | SC 10% | 34.9 b…h | 13.5 c…g | 8.5 abc | 8.5 a |
| | Willow 5% | 23.5 g…k | 5.9 h…l | 7.0 a…g | 2.9 h…k |
| | Willow 10% | 15.2 j…n | 0.9 l | 4.6 hjk | 1.3 kl |
| **Sunflower** | Control | 39.7 a…f | 10.7 e…k | 8.5 abc | 4.3 efg |
| | Buckwheat 5% | 44.9 a…d | 12.1 d…i | 8.9 a | 4.5 def |
| | Buckwheat 10% | 32.9 c…i | 11.2 e…j | 6.8 a…i | 5.4 cd |
| | SC 5% | 49.1 a | 20.4 ab | 8.4 a…d | 5.9 c |
| | SC 10% | 46.3 abc | 27.0 a | 6.7 b…i | 6.7 bc |
| | Willow 5% | 41.5 a…e | 10.0 e…k | 8.7 ab | 5.0 cde |
| | Willow 10% | 22.4 h…l | 13.5 c…g | 5.9 e…j | 5.0 cde |

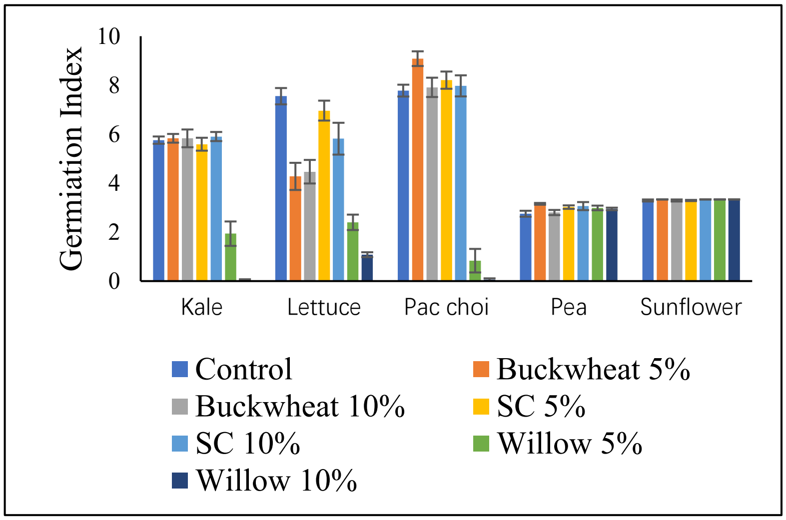
**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.2 The average germination rate (%) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

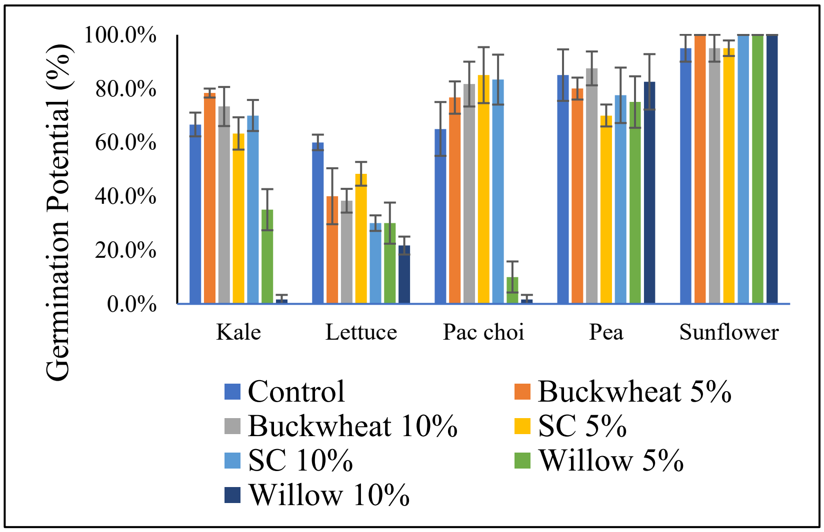
Seed germination rate is the most direct parameter to evaluate the viability of a population of seeds (Yang et al., 2015). For lettuce, the average germination rate of seeds treated with both the 5% willow HTCPL and 10% willow HTCPL were significantly lower than others (Figure 4.1 & Figure 4.2). Among all sample solutions, the 5% SC HTCPL had the best promotion effect, but it was no different from the control. The willow HTCPLs did not seem suitable for the kale and the pac choi seeds germination (Figure 4.1 & Figure 4.2). The seeds germination rate of kale and pac choi with 5% willow HTCPL or the 10% willow HTCPL was significantly lower than the seeds with other treatments (Figure 4.1 & Figure 4.2). There was no significant difference in the effects of various treatments on the germination rate of the sunflower and the pea seeds (Figure 4.1 & Figure 4.2). In terms of germination rate, the HTCPL samples did not show superior results for the seeds compared to the control. This may be because seed germination does not require fertilizer, and some mineral components in individual HTCPLs may not be conducive to seed germination.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.3 The germination index of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

It is not sufficient to use only seed germination rate to show seed germination activity (Yang et al., 2015). Germination index is a good parameter to describe the relationships between the germination rate and germination speed (Yang et al., 2015). In terms of the lettuce, kale and pac choi, the germination index of all other treatments was significantly higher than the 5% willow HTCPL and the 10% willow HTCPL (Figure 4.1 & Figure 4.3). Compared with all the HTCPL solutions, water (control) showed higher germination index for lettuce (Table 4.4). There were no significant differences in both sunflower's and pea's germination index between different treatments. The seeds of lettuce, kale, and pac choi are smaller than the seeds of sunflower and pea (Figure 4.1 & Figure 4.3). The willow HTCPLs exhibited a significantly negative effect on the germination index of the smaller size seeds in this study (Figure 4.3).

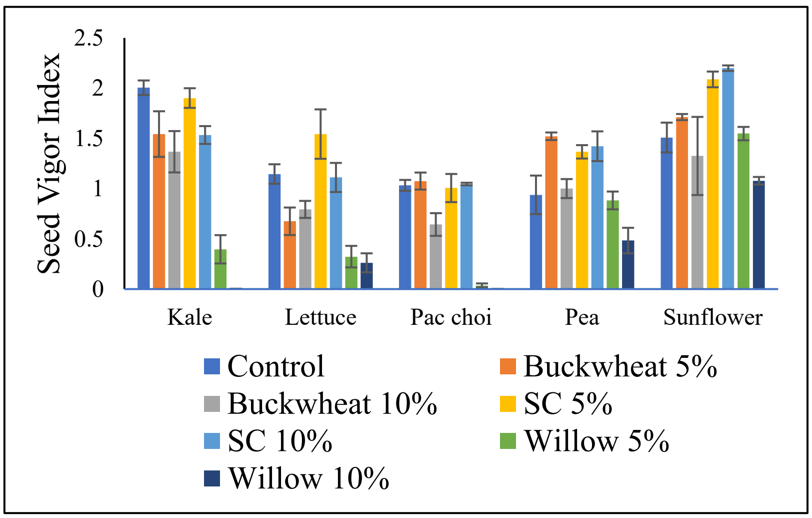



*Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
*SC, seafood compost*
*Willow, willow (Salix babylonica) branches*

Figure 4.4 The average germination potential (%) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

Germination potential provides an evaluation of the field performance of seeds (Yang et al., 2015). In this study, there was no significant difference in the effects of various treatments on the germination potential of lettuce, sunflower and pea seeds. In terms of kale and pac choi, the results of germination potential were similar. In addition to the 5%

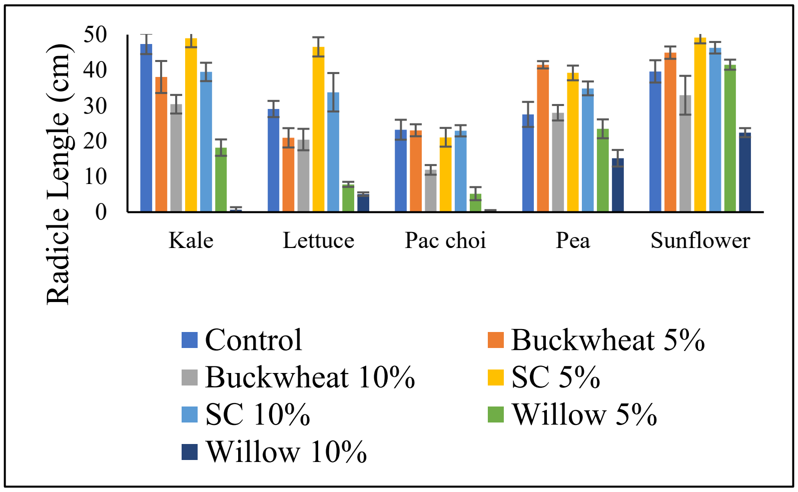
willow HTCPL, the seeds applied with other sample solutions and the control had significantly higher germination potential than the seeds applied with the 10% willow HTCPL (Figure 4.1 & Figure 4.4). The results suggested that HTCPL samples did not promote the germination potential of seeds. Moreover, the effect of HTCPLs on the field performance of seeds was related to the species of seeds.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.5 The seed vigor index of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

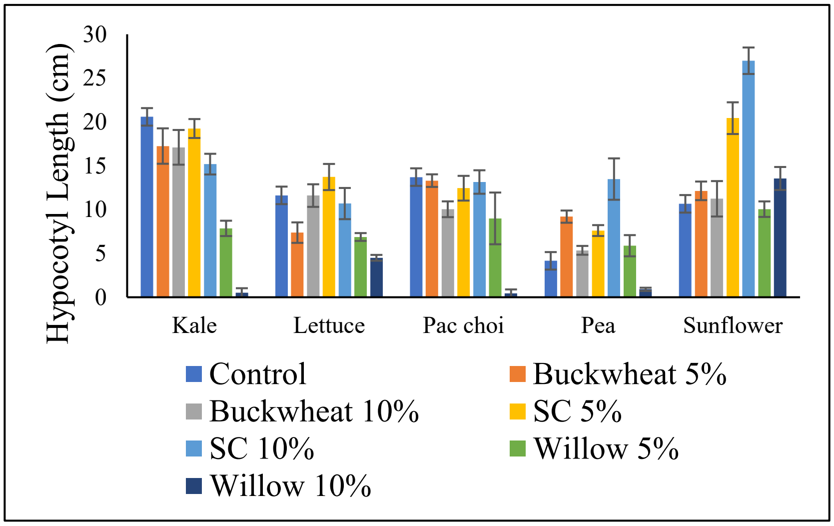
In addition to germination rate, germination potential and germination index, the seed vigor index is an important parameter, which reflects the sum of seed characteristics that determine the activity level and performance of seed germination and emergence (Yousefi el al., 2017). The lettuce seeds applied with the willow HTCPLs had the lowest seed vigor index (Table 4), and the seed vigor index of them was significantly lower than the lettuces grew with the 5% SC HTCPL (Figure 1 & 4). The performances of the willow HTCPLs on the kale, pac choi and pea were among the worst (Table 4.4). For the kale, the performance of other sample solutions and the control were significantly better than the 5% willow HTCPL and the 10% willow HTCPL. In terms of the pac choi, in addition to the seeds treated with the 10% buckwheat HTCPL, the seed vigor index of other treated seeds was significantly higher than the seeds applied with either the 5% willow HTCPL or the 10% willow HTCPL (Figure 4.1 & Figure 4.5). The 5% buckwheat HTCPL achieved the highest seed vigor index for the pea, although the 5% buckwheat HTCPL had no significant difference from other samples and the control except that it was significantly better than the 10% willow HTCPL (Table 4.3, Figure 4.1 & Figure 4.5). For the sunflower, the seeds treated with the 10% SC HTCPL showed the highest seed vigor index (Table 4), which was significantly higher than the seed vigor index of the seeds grew in the 10% willow HTCPL and the 10% buckwheat HTCPL germination pouches (Figure 4.1 & Figure 4.5). From the results, it is obvious that willow HTCPLs caused a negative effect on seed vigor index, but there was no individual HTCPL treatment that showed excellent affects compare to the control.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.6 The average radicle length (cm) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

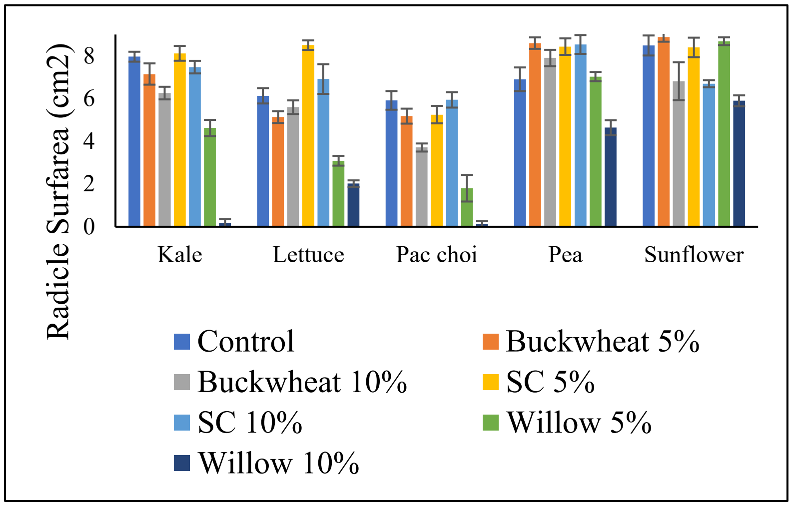
The kales applied with the 5% SC HTCPL had the highest average radicle lengths (Table 4.4). However, there was no significantly different effect between the 5% SC HTCPL and the control (Figure 4.1 & Figure 4.6). The willow HTCPLs showed a negative effect on radicle lengths' growth of kale (Figure 4.1). In addition to the kale grew in the germination pouches with the 10% buckwheat HTCPL, all the kales applied with other sample solutions made kales' radicle lengths were significantly higher than that treated with the 5% willow HTCPL and the 10% willow HTCPL (Figure 4.1 & Figure 4.6). Compared with the control, the 5% SC HTCPL exhibited the significant promoted effect on the growth of lettuces' radicle lengths, while the 5% willow HTCPL and the 10% willow HTCPL showed the significant negative effect on the growth of lettuces' radicle lengths (Figure 4.1 & Figure 4.6). In terms of the pac choi, the SC HTCPLs did not present a prominent impact. Besides the 10% buckwheat HTCPL and the 5% willow HTCPL, all of the sample solutions significantly increased the growth of pac chois' radicle lengths compared to the 10% willow HTCPL (Figure 4.1 & Figure 4.6). The growth of the radicle lengths of sunflower was similar to the pac choi, in addition to the 10% buckwheat HTCPL, all of the sample solutions exhibited the significant positive effect on the growth of sunflowers' radicle lengths compared to the 10% willow HTCPL. For the pea, the superior impact appeared on the 5% buckwheat HTCPL, which was significantly higher than the control, the 5% willow HTCPL and the 10% willow HTCPL (Figure 4.1 & Figure 4.6). In short, compared with the control, the HTCPL samples did not show a significant positive effect on the growth of radicle lengths of kale, pac choi, and sunflower. However, for the lettuce and pea, the promotion effect of individual samples was significantly higher than that of the control. The lengths growths of lettuces' radicles were more sensitive to the HTCPLs than others (Figure 4.6).




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.7 The average hypocotyl length (cm) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

For pac choi, kale, and pea, the shortest average hypocotyl lengths appeared in the young crops treated with the 10% willow HTCPL (Table 4). The average hypocotyl length of pac choi applied with all treatments were significantly higher than that treated with the 10% willow HTCPL, and there was no significant difference among all treatments besides the 10% willow HTCPL (Figure 4.1 & Figure 4.7). The kales applied with the 10% SC HTCPL had the highest average hypocotyl lengths (Table 4.4), which was significantly higher than the kales grew in the germination pouches with the 5% willow HTCPL and the 10% willow HTCPL (Figure 4.1 & Figure 4.7). However, there was no significant difference between the 10% SC HTCPL and the other treatments, including the control (Figure 4.1 & 4.7). For the pea, the longest average hypocotyl length was also presented on the plants treated with the 10% SC HTCPL (Table 4.4), and the positive effect of the 10% SC HTCPL was significantly higher than the control, the 5% willow HTCPL, the 10% buckwheat HTCPL and the 10% willow HTCPL (Figure 4.1 & 4.7). The hypocotyl lengths of lettuces applied with the 5% SC HTCPL were significantly longer than the lettuces applied with the 10% willow HTCPL, but the effect of the 5% SC HTCPL was not significantly different from that of other treatments (Figure 4.1 & Figure 4.7). In terms of the sunflower, the shortest hypocotyl lengths appeared in the sunflowers grew on the 5% willow HTCPL germination pouches (Table 4.4). The optimal HTCPL solutions for the growth of sunflowers' hypocotyl lengths were the 5% SC HTCPL and the 10% SC HTCPL because their promotion effects were significantly higher than the other HTCPL solutions and the control (Figure 4.1 & Figure 4.7). Overall, it is obvious that the willow HTCPL was the least suitable for hypocotyl lengths development and growth.

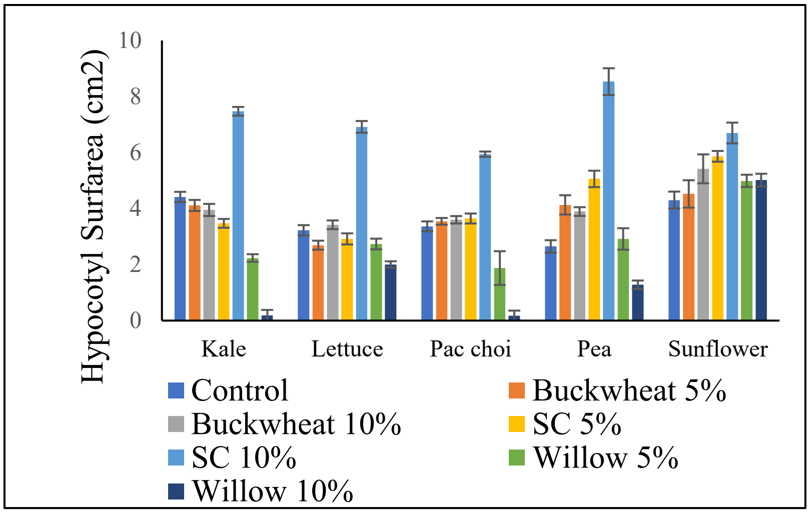



**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 4.8 The average radicle surface area ($cm^2$) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (*Brassica oleracea var. sabellica*), and lettuce (*Lactuca sativa*) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

Radicle surface area is closely related to the absorption of water and nutrients by seedlings. The lettuces grew with the 5% SC HTCPL showed the largest average radicle surf-area growths (Table 4.4), although there was no significantly different effect between the 5% SC HTCPL and the control (Figure 4.1 & Figure 4.8). The average radicle surface area of lettuces watered with the willow HTCPLs were significantly smaller than the lettuces applied with other sample solutions. For both kale and pac choi, the effects of the willow HTCPLs were worst (Table 4.4, Figure 4.1 & Figure 4.8). In addition to the 5% willow HTCPL, all other treatments performed significantly better than the 10% willow HTCPL.

It seems that the 10% buckwheat HTCPL was not suitable for the development of radical surface area of kale and pac choi (Figure 4.1 & Figure 4.7). In addition to the 10% buckwheat HTCPL and the 10% willow HTCPL, the average radicle surface area of the seedlings cultivated by all other treatments were significantly larger than those cultivated by the 5% willow HTCPL (Figure 4.1 & Figure 4.7). Although the 5% willow HTCPL did not work well on the objects of lettuce, kale, and pac choi, it did not exhibit the negative effect on sunflowers and pea. The sunflowers applied with the 5% buckwheat HTCPL, the 5% willow HTCPL, the 5% SC HTCPL and the control had significantly larger average radicle surface area than the sunflowers applied with the 10% willow HTCPL (Figure 1 & Figure 7). Moreover, the 5% buckwheat HTCPL significantly increased the average radicle surface area of sunflower compared to the 10% SC HTCPL (Figure 4.1 & Figure 4.7). The average radicle surface area of the peas treated with the 10% willow HTCPL were significantly smaller than other treated peas, and there was no significant difference in the average radicle surface area among treatments (Figure 4.1 & Figure 4.7). Compared with the control, for the five plants used in the experiment, it seems that none of the HTCPL samples has a significant promoting effect, but the willow HTCPL has shown a significant negative effect.

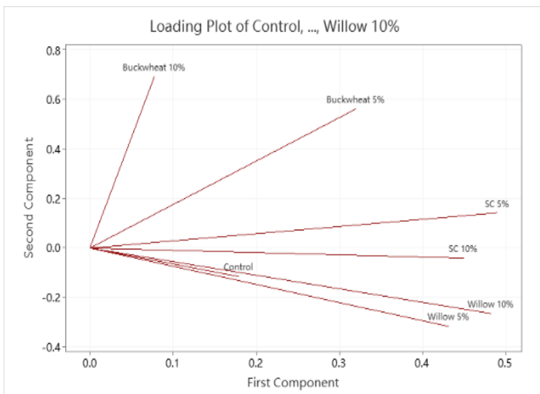



**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

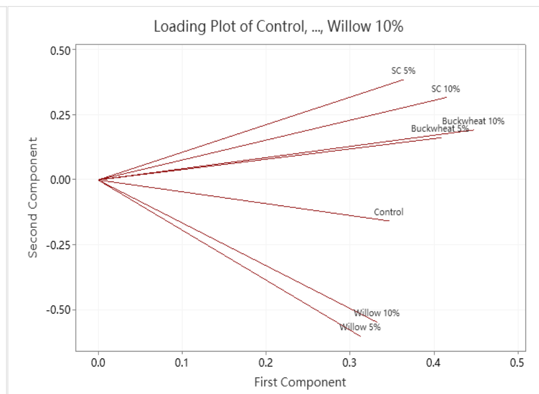
Figure 4.9 The average hypocotyl surface area (cm$^2$) of pea (*Pisum sativum*), sunflower (*Helianthus annuus*), pac choi (*Brassica rapa subsp. chinensis*), kale (Brassica oleracea var. sabellica), and lettuce (*Lactuca sativ*a) treated with different HTCPL (hydrothermal carbonization processed liquid) samples.

The average hypocotyl surf-area of both sunflowers and peas grew on the 10% SC HTCPL germination pouches was highest (Table 4.4). For the sunflower, the positive effect of the 10% SC HTCPL was significantly better than the control and the 10% buckwheat HTCPL (Figure 4.1 & Figure 4.8). The average hypocotyl surface area of the peas applied with the 10% SC HTCPL was significantly higher than all other treated peas except the 5% buckwheat HTCPL and the 5% SC HTCPL (Figure 4.1 & Figure 4.8). In addition to the control, all treated peas had significantly larger average hypocotyl surface area than the plants applied with the 10% willow HTCPL (Figure 4.1 & Figure 4.8). The 10% willow HTCPL was not only had the worst impact on the pea, but also kale and pac choi (Table

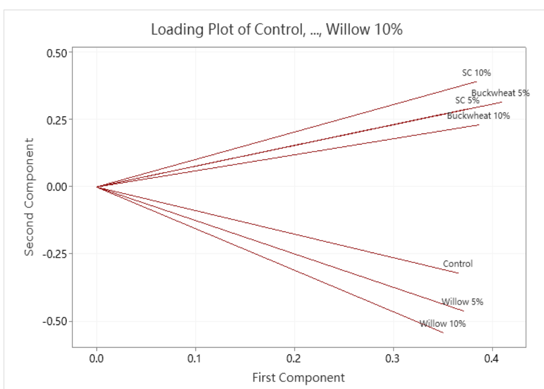
4.4). In terms of both kale and pac choi, the average hypocotyl surface area of all treated plants was significantly larger than that applied with the 10% willow HTCPL, and the effect of most treatments was better than the 5% willow HTCPL (Figure 4.1 & Figure 4.8).



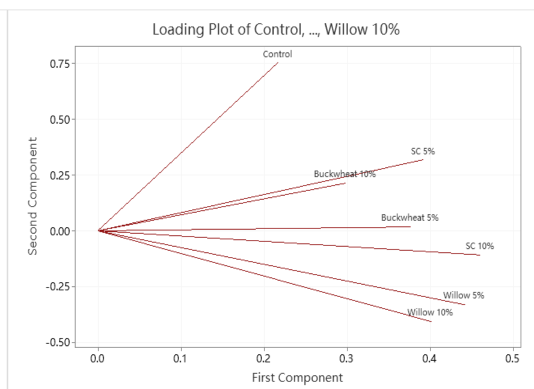

(a)




(b)

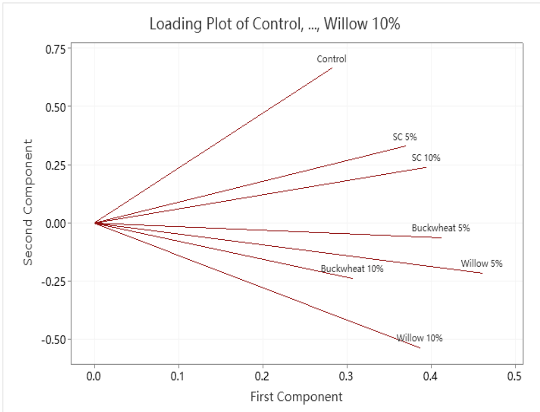


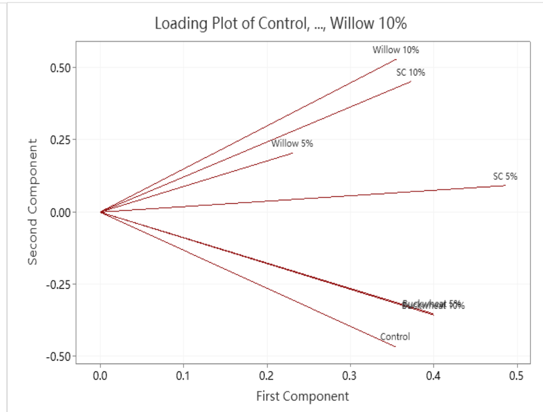

(c)




(d)

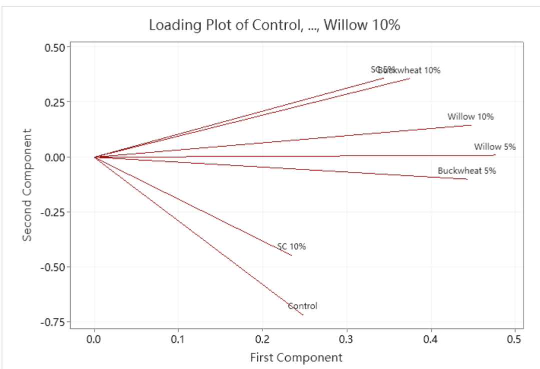
**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

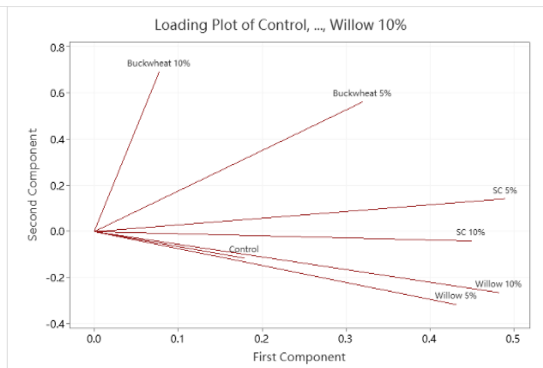



(e)

(f)




(g)

(h)

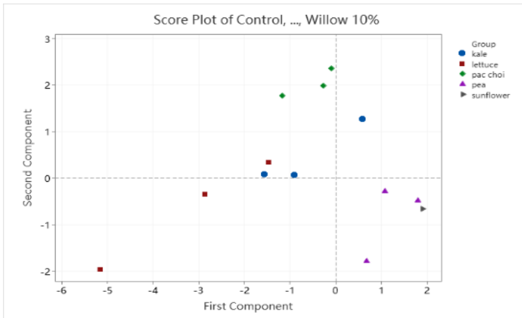
**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*

**HTCPL, hydrothermal carbonization processed liquid*

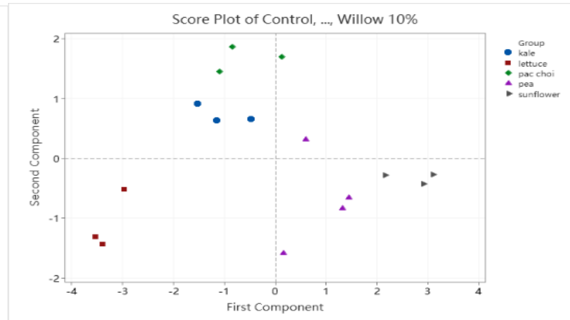
**SC, seafood compost*

**Willow, willow (Salix babylonica) branches*

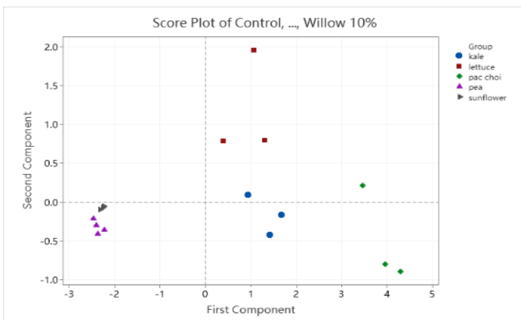
Figure 4.10 The PAC loading plot for the first two components of average germination rate (%) (a), germination potential (%) (b), germination index (c), seed vigor index (d), radicle length (cm) (e), hypocotyl length (cm) (f), radicle surface area (cm2) (g) and hypocotyl surface area (cm2) (h).



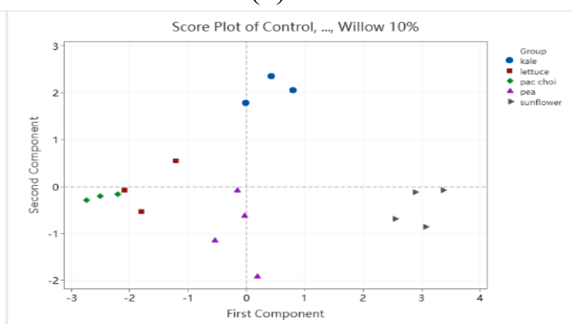

(a)



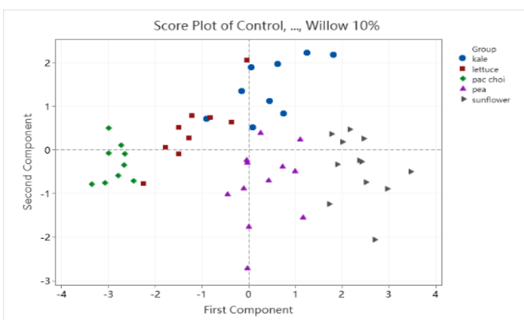

(b)



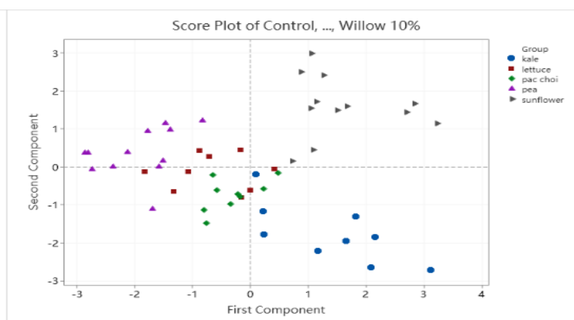

(c)



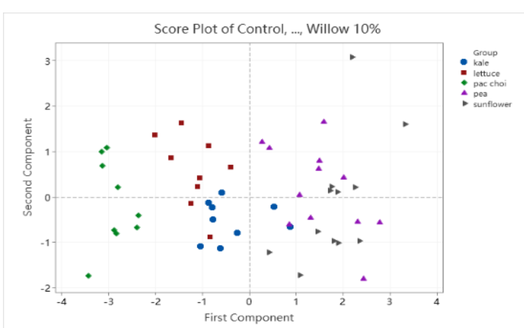

(d)



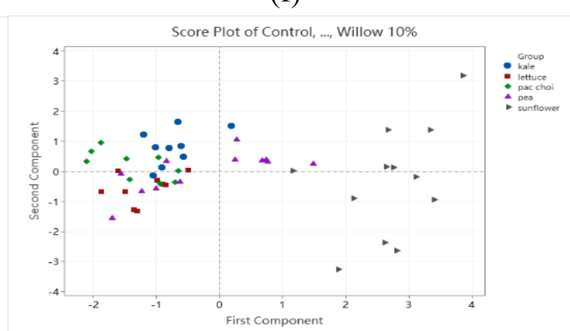

(e)




(f)




(g)




(h)

** kale (Brassica oleracea var. sabellica)*

** pac choi (Brassica rapa subsp. Chinensis)*

** lettuce (Lactuca sativa)*

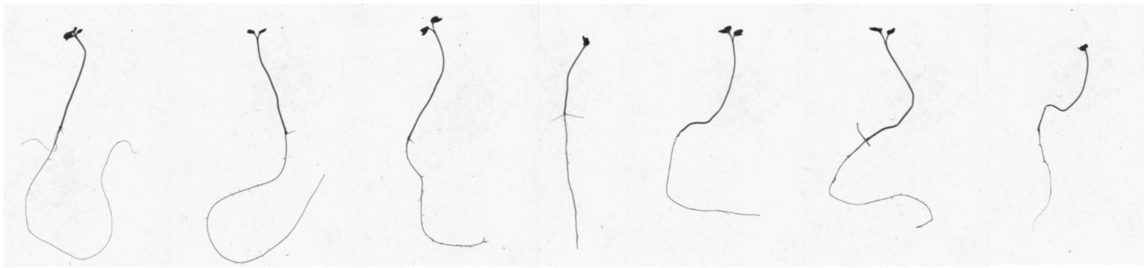
* *pea (Pisum sativum)*
* *sunflower (Helianthus annuus)*

Figure 4.11 The PCA (principal components analysis) Score plot for the first two components of average germination rate (%) (a), germination potential (%) (b), germination index (c), seed vigor index (d), radicle length (cm) (e), hypocotyl length (cm) (f), radicle surface area (cm2) (g) and hypocotyl surface area (cm2) (h).

Treatments had different loading on the first two components of average germination rate (%) germination index, germination potential (%), seed vigor index, radicle length (cm), hypocotyl length (cm), radicle surf-area ($cm^2$) and hypocotyl surf-area ($cm^2$) (Figure 4.10). For example, the 5% Buckwheat HTCPL and the 10% Buckwheat HTCPL have large positive loading on the first component on hypocotyl length (cm) but these two treatments have large positive loading on the second component on hypocotyl surface area ($cm^2$). Therefore, in terms of different measuring data, the first two components measure different factors. From the score plots graph (Figure 4.11), it is obvious that the five species (kale, sunflower, pea, pac choi and lettuce) showed different loading on the first two components. Taking radicle length as the example, the group of sunflowers was on the right-hand side of the plot, the majority group of pea and kale were on the right-hand side of the plot, the group of lettuce and pac choi were on the left-hand side of the plot. In addition, one species has different loading on the first two components on the different measuring data. For instance, the majority group of kale was on the right-hand side of the plot of radicle length (cm), but the majority of this group was on the left-hand side of the plot of hypocotyl length (cm). Although principal components analysis (PCA) results cannot clearly describe the factors affecting seed germination and young crop growth, the results of PCA in this experiment, show that different species were affected to varying degrees by HTCPL samples.

Experiment 2 - *Seed germination and seedling growth of kale, response to 0.5%, 1%, and 2.5% HTCPLs from different biomass*

The results of experiment 1 indicated that the smaller size of seeds, including kale, pac choi, and lettuce were more sensitive to the HTCPLs (Table 4.4 or Figure 4.1). The effects of SC HTCPLs and buckwheat HTCPLs were similar. Both of them did not show a significant effect on seed germination and seedling growth. The willow HTCPLs had a significantly negative effect on seed development, and the low pH value may be the reason. Therefore, based on the above results from experiment 1, experiment 2 readjusted the treatments and objects for deeper understanding the impact of HTCPL on seed germination and seedling growth.



(a) (b) (c) (d) (e) (f) (g)

**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

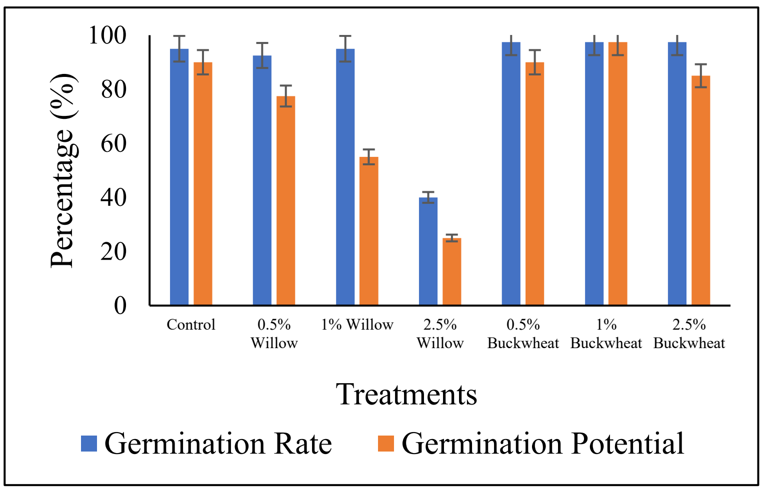
Figure 4.12 The morphology of kale (*Brassica oleracea var. sabellica*) in the treatments of control (a), 0.5% willow HTCPL (hydrothermal carbonization processed liquid) (b), 1% willow HTCPL (c), 2.5% willow HTCPL (d), 0.5% buckwheat HTCPL (e), 1% buckwheat HTCPL (f), and 2.5% buckwheat HTCPL (g).

Table 4.5 The average germination rate (%), germination index, germination potential (%), seed vigor index, radicle length (cm), hypocotyl length (cm), radicle surface area (cm2) and hypocotyl surface area (cm2) of kale (*Brassica oleracea var. sabellica*) treated with the HTCPL (hydrothermal carbonization processed liquid) samples.

| **Treatments** | **Germination Rate (%)** | **Germination Potential (%)** | **Germination Index** | **Seed Vigor Index** |
|---|---|---|---|---|
| **Control** | 95.0 a | 90.0 a | 9.1875 a | 0.511699 a |
| **0.5% Willow HTCPL** | 92.5 a | 77.5 a | 8.3125 ab | 0.522472 a |
| **1% Willow HTCPL** | 95.0 a | 55.0 b | 7.00833 b | 0.524871 a |
| **2.5% Willow HTCPL** | 40.0 b | 25.0 c | 2.34167 c | 0.172176 b |
| **0.5% Buckwheat HTCPL** | 97.5 a | 90.0 a | 9.3 a | 0.513446 a |
| **1% Buckwheat HTCPL** | 97.5 a | 97.5 a | 9.75 a | 0.452544 a |
| **2.5% Buckwheat HTCPL** | 97.5 a | 85.0 a | 9.125 a | 0.454599 a |
| **Treatments** | Radicle Length (cm) | Hypocotyl Length (cm) | Radicle Surface area (cm2) | Hypocotyl Surface area (cm2) |
| **Control** | 31.241 ab | 22.6446 ab | 5.52450 ab | 5.65785 ab |
| **0.5% Willow HTCPL** | 33.7843 a | 22.7491 ab | 6.15950 a | 5.4229 ab |

| **Treatments** | Radicle Length (cm) | Hypocotyl Length (cm) | Radicle Surface area (cm2) | Hypocotyl Surface area (cm2) |
|---|---|---|---|---|
| **1% Willow HTCPL** | 27.0998 abc | 27.9918 a | 5.32448 ab | 6.4516 a |
| **2.5% Willow HTCPL** | 24.736 abc | 18.6109 b | 4.75932 ab | 4.7117 b |
| **0.5% Buckwheat HTCPL** | 25.9539 abc | 26.5140 ab | 4.82600 ab | 6.44842 a |
| **1% Buckwheat HTCPL** | 22.2246 bc | 24.1910 ab | 4.98793 ab | 6.05155 ab |
| **2.5% Buckwheat HTCPL** | 20.9639 c | 25.6704 ab | 4.30848 b | 5.99758 ab |

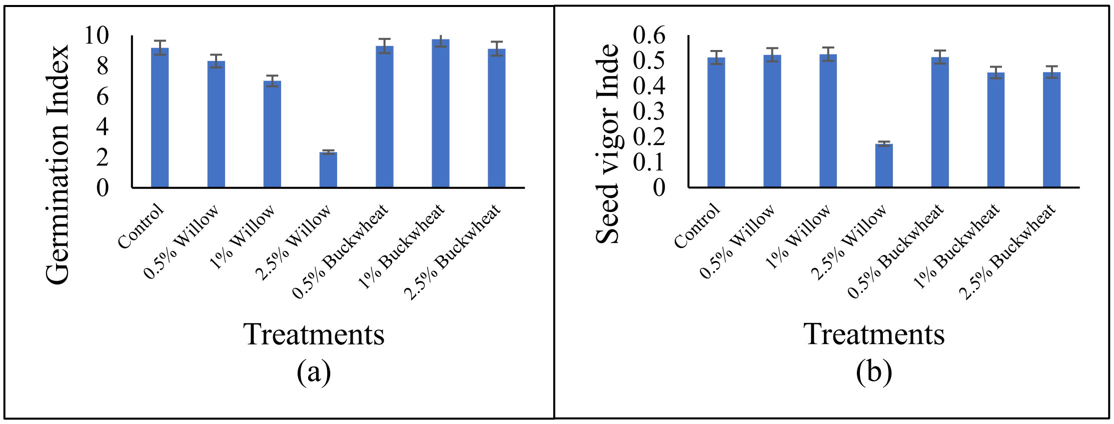
**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**Willow, willow (Salix babylonica) branches*




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**Willow, willow (Salix babylonica) branches*

Figure 4.13 The average germination rate (%) and germination potential (%) of kale (*Brassica oleracea var. sabellica*) treated with the HTCPL (hydrothermal carbonization processed liquid) samples.

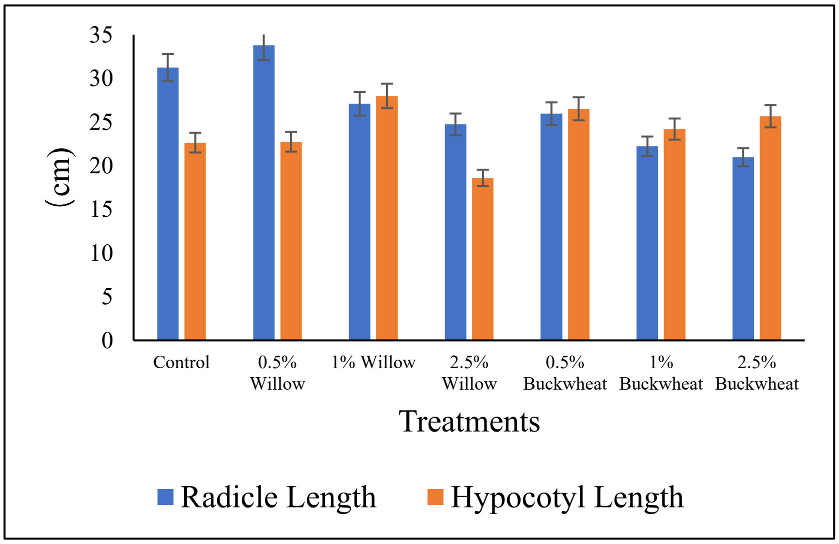
It is obvious that the germination rate and the germination potential of kale seeds treated with the 2.5% willow HTCPL were significantly lower than that of other treatments (Table 4.5 & Figure 4.13). The germination potential of 1% willow HTCPL treatment was also low at 55% (Table 4.5 & Figure 4.13), which indicated that the filed performance of seeds applied with 1% willow HTCPL may not be promising.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**Willow, willow (Salix babylonica) branches*

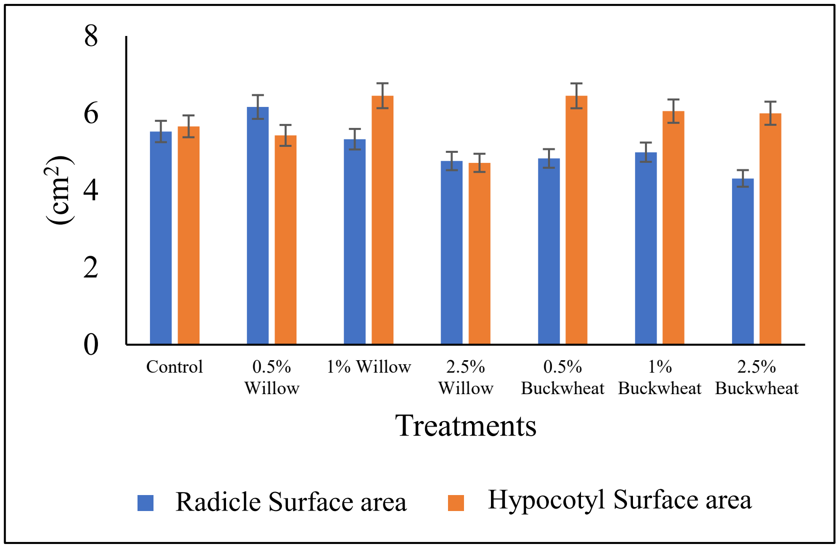
Figure 4.14 The average germination index (a) and average seed vigor index (b) of kale (*Brassica oleracea var. sabellica*) treated with the HTCPL (hydrothermal carbonization processed liquid) samples.

Germination index describes the relationships between the germination rate and germination speed (Yang et al., 2015), and seed vigor index reflects the activity level and performance of seed germination and emergence (Yousefi el al., 2017). The kale seeds treated with 2.5% willow HTCPL had the least value of germination index and seed vigor index (Table 4.5 & Figure 4.14). Obviously, the lowest germination rate, germination potential, germination index, and seed vigor index were apparent in kale applied with the 2.5% willow HTCPL (Table 4.5 & Figure 4.14).




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**Willow, willow (Salix babylonica) branches*

Figure 4.15 The average radicle length and hypocotyl length of kale (*Brassica oleracea var. sabellica*) treated with the HTCPL (hydrothermal carbonization processed liquid) samples.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**Willow, willow (Salix babylonica) branches*

Figure 4.16 The average radicle surface area and hypocotyl surface area of kale (*Brassica oleracea var. sabellica*) treated with the HTCPL (hydrothermal carbonization processed liquid) samples.

In terms of radicle length, the highest and lowest values were presented in the kales treated with the 0.5% willow HTCPL and the 2.5% buckwheat HTCPL, respectively (Table 4.5 & Figure 4.15). The kales applied with the 1% willow HTCPL had the highest hypocotyl length. Hypocotyl length of kale plant treated with 2.5% willow HTCPL was significantly lower than that of in the other treatments (Table 4.5 & Figure 4.15). The 2.5% buckwheat HTCPL lead to kale plant that had the smallest radicle surface area and the 0.5% willow HTCPL had the largest radicle surface area, which was 4.31 $mm^2$ and 6.12 $mm^2$, respectively (Table 4.5 & Figure 4.15). The smallest hypocotyl surface area and largest hypocotyl surface area appeared in kales applied with the 2.5% willow HTCPL and the 1% willow HTCPL, respectively (Table 4.5 & Figure 4.16). In short, it implies that the appropriate concentration of HTCPL may be beneficial to the growth of the seedling, but when the concentration of HTCPL exceeds a certain range, the concentration of HTCPL and the growth of the seedling were negatively correlated.

## 4.5. Discussion

Germination rate, germination potential, germination index, and seedling vigor index are parameters that reflect seed germination. According to the results of Experiment 1, sunflower and pea were less sensitive to HTCPL than kale, lettuce and pac choi. Compared with sunflower and pea, the seed sizes of kale, lettuce and pac choi are smaller, and studies have shown that seed size is an important factor affecting seed germination (Akinyosoye et al, 2014). The larger the seed size, the less the interference of external factors on seed germination because the available substrates and energy (ATP), and the synthesis efficiency of active enzymes and proteins increased with the increase of seed size (Rynd, 1978; Özgen et al., 2007). Therefore, it is suggested that the size of the seed and the genetic characteristics of the seed cause the seeds to respond differently to treatments.

The Experiment 1 results showed that the willow HTCPLs inhibited seed germination, while SC HTCPLs and the buckwheat HTCPLs did not significantly promote seed germination compared with control. Seeds do not need fertilizer for germination and emergence since they depend on the internal nutrients for germination (Milberg et al., 1998; Vaughton & Ramsey, 2001). Some studies have shown that in a certain range, the change of medium pH value will not lead to a significant impact on seed germination (Roem et al., 2002; Koger et al., 2004). The optimal pH range for the different species may be different, but the starting value for requiring by seed is generally over 5 (Roem et al., 2002; Koger et al., 2004). The pH values of the 5% willow HTCPL and the 10% willow HTCPL were the least among all treatments i.e., 4.04 and 3.75, respectively (Table 4.3), and they may not be within the pH range suitable for seed germination. Therefore, the willow HTCPL solutions were not conducive for seed germination, which may be due to high acidity rather than mineral composition and content. In fact, the Experiment 2 results also confirm the above conjecture. In Experiment 2, the pH value of the 2.5% willow HTCPL was the lowest, and the various germination parameters including germination rate, germination potential, germination index, and seed vigor index, of the kales applied with it were also the lowest (Table 4.3 & Table 4.4).

Radicle length, plumule length, radicle surface area, and plumule surface area are growth parameters, which are positively correlated with seedling growth. Compared with the control treatments, HTCPLs did not seem to have a clear advantage in the development of seedling, because HTCPLs did not increase the radicle length, plumule length, radicle surface area, and plumule surface area of object plants significantly. The supply of nitrogen, potassium, magnesium, phosphorus, iron, calcium and other nutrients affected the growth of seedlings. Nitrogen, phosphorus and potassium are usually the main influential macronutrients of plants but the nature and degree of response varies from one plant species to the other (Hegarty,1976; Fenner & Lee, 1989; Hanley & Fenner, 1997). Either nutritional deficiency or excess is not conducive to the growth of seedlings (Hegarty,1976; Fenner & Lee, 1989; Hanley & Fenner, 1997). From the results of HTCPL mineral elements analysis, the contents of phosphorus and potassium in the SC HTCPLs were significantly lower than that in the willow HTCPLs and the buckwheat HTCPLs (Table 4.2). Moreover, the content of harmful elements in the HTCPLs can also affect the growth of plants. It can be observed that the lead content in the SC HTCPLs was the lowest, while that in willow HTCPLs was the highest (Table 4.2). However, from the results of Experiment 1, there was no obvious difference between the effects of SC HTCPLs and buckwheat HTCPLs on seed germination and seedling growth (Table 4.4). Using buckwheat HTCPL and willow HTCPL to cultivate plants may be more promising for increased productivity than SC HTCPL.

This work suggested that growing seedlings with a lower concentration of HTCPLs may achieve more ideal results. When the pH is below the suitable range (generally 5.5-7.0) for plant growth, the respiration of plant roots, ion exchange, and absorption of various nutrients will be hindered (McKee 1961; Melhuish et al., 1990; Liu & Hanlon, 2015). It has been found that phosphorus limitation caused by low pH is one for the main reasons of inhibiting the growth of seedlings (Hegarty,1976). Iron, copper, zinc and other elements in acid solution combine with phosphate to form precipitates that lead to phosphorus limitation (Hegarty, 1976). However, phosphorus limitation does not seem to be the reason why the high concentrations of willow HTCPLs (5% and 10%) were not suitable for seedling growth. Because the promotion effect of the 5% and 10% willow HTCPLs on seedlings were obviously lower than that of water alone (control), and no obvious precipitation was observed in all the different types of HTCPL solutions used in the present study. A previous study has shown that beans have a high demand for calcium (Fenner & Lee, 1989), so it is speculated that pea may be more sensitive to the calcium content in solutions compared to kale, lettuce, pac choi and sunflower. However, the experimental results do not confirm this conjecture. In short, combining Experiment 1 and Experiment 2, HTCPL from the different sources of biomass did not significantly promote seed germination and seedling growth, which may be related to the fact that seed development does not require fertilizer. Whether lower concentration HTCPL can be used as a promoter of seed germination and seedling growth remains to be studied.

### 4.6. Conclusion

This research project focuses on the assessment of the efficacies of the HTCPLs from three different biomass, namely; willow branches, seafood compost, and buckwheat green foliage, on seed germination and seedling growth of pea, sunflower, kale, pac choi and lettuce. Overall, HTCPLs did not show significant promoting effect on seed germination and seedling growth. On the contrary, a high concentration of willow HTCPL may significantly inhibit seed germination, as a result of its low pH value. Until now, there are only a few studies explicitly evaluating the effect of HTCPL on seed germination and seedling growth. As such, more and better investigation will be required to confirm the results and efficacy of HTCPL.

# Chapter 5 Pea Baby Greens Growth and Mineral Element Composition Response to Hydrothermal Carbonization Processed Liquids Extracted from Different Biomasses

## 5.1 Abstract

In this work, the plant growth parameters and mineral contents of pea (*Pisum sativum* L.) baby greens treated with the different concentrations of Hydrothermal Carbonization Processed Liquids (HTCPLs) were evaluated. The HTCPLs were obtained from willow (*Salix babylonica*) branches, buckwheat (*Fagopyrum esculentum*) green foliage and seafood compost (SC) by using 200 ºC extraction temperature. Regardless of feedstock, high concentrations (5% and 10%) of HTCPLs were not suitable for the growth of pea baby greens. However, the effects of the low concentrations (0.5%, 1%, 2.5%) of HTCPLs and the control (Pro-Mix BX$^{TM}$) on the growth of pea baby greens were generally similar. It is suggested that HTCPL extracted from different biomass has potential to be used as a bio-fertilizer to growing baby greens. The mineral composition of the pea baby greens treated with HTCPL samples reached the standard range of mineral composition of baby greens.

*Keywords: waste product, hydrothermal carbonization, bio-fertilizer, baby greens,*

## 5.2 Introduction

Baby greens become a new superfood in recent decades. Aromatic flavors, rich colors and texture and high nutritive value are the characteristics of baby green (Francis et al. 2012; Baselice et al., 2017; Lenzi et al., 2019; Di Bella et al., 2020). As immature plant, the growth cycle of baby greens is usually short i.e., between 20-40 days, and the growth process requires fertilizer and light (Di Gioia et al., 2017). Generally, most of baby greens are harvested after the true leaf development (Di Gioia et al., 2017). Pea (*Pisum sativum* L.) is a rich high-protein plant, and the part that is often eaten is its seeds (Merwad, 2018). In recent years, eating pea baby greens has become more popular. The reason for its popularity is mainly due to its richness in micronutrients including vitamin C, vitamin E, vitamin A and potassium (Santos et al., 2014). Previous studies have proved that the leaves of pea baby greens contain significant proportion of flavonoids, which can prevent chronic diseases in humans (Santos et al., 2014).

Hydrothermal carbonization processed liquid (HTCPL) is a by-product of the hydrothermal carbonization process (HTC), which has the potential to play the role of bio-fertilizer or growth stimulator on plants (Sun et al., 2014; Funke, 2015; Yao et al., 2016). Many organic biomasses can be used as feedstock for HTC such as various plants, composts, and even sewage sludge (Saetea & Tippayawong, 2013; Funke, 2015; Idowu et al., 2017; Yao et al., 2016). The composition and properties of HTCPL are affected by the feedstocks of HTC, heating time, heating temperature, pressure and other conditions, and the feedstocks have the largest impact on the composition and property of HTCPL (Saetea & Tippayawong, 2013; Funke, 2015; Idowu et al., 2017). Previous studies have shown that HTCPL retains most of the original nitrogen and can be used as nitrogen fertilizer (Saetea & Tippayawong, 2013; Sun et al., 2014). However, some studies are skeptical about the effectiveness of HTCPL as fertilizer (Sun et al., 2014; Idowu et al., 2017).

The hypothesis of this study is that HTCPL obtained from different biomasses can be used as a fertilizer for young crop. Therefore, the objective of this study is to investigate pea baby greens growth and mineral element composition response to HTCPL obtained from willow (*Salix babylonica*) branches, seafood compost, and buckwheat (*Fagopyrum esculentum*) green foliage.

## 5.3 Material and Methods

### 5.3.1 Location and materials

The HTCPL extraction was produced in the lab of the Faculty of Sustainable Design Engineering, University of Prince Edward Island (UPEI) from May 2019 to August 2019. The experiments of pea baby greens growth and mineral quality response to HTCPL were performed at the Department of Plant, Food, and Environmental Sciences, Dalhousie University Agricultural Campus (45.37°N, 63.26°W) from May 2020 to August 2020. The mineral element compositional analysis of pea baby greens was done at the PEI Analytical Laboratories (Charlottetown, PEI) in August 2021. the Seafood compost (Greenhouse Gold™, NB, Canada) was purchased from PEI retailers, and willow branches and buckwheat green foliage were obtained from the experimental field of UPEI and experimental field of Dalhousie Agriculture campus, respectively. Organically certified pea seeds were purchased from West Coast Seed™ (BC, Canada); and rockwool cubes from Canna (Toronto, Canada) was from Halifax Seeds Inc., NS. HTCPLs used in this work were obtained from the three biomasses by using 200 ºC reacting temperature.

Table 5.1 Feedstocks and process conditions used to produce HTCPL from willow (*Salix babylonica*) branches, SC, and buckwheat (*Fagopyrum esculentum*) green foliage

| **Feedstock** | **Willow branches** | **SC** | **Buckwheat green foliage** |
|---|---|---|---|
| **Process temperature ºC** | 200 | 200 | 200 |
| **Initial pressure psi** | 100 | 100 | 100 |
| **Process heating rate ºC/min** | 4.9 | 4.9 | 4.9 |
| **Process time min** | 60 | 60 | 60 |

**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*

Table 5.2 PH value of the different HTCPL samples.

| | 0.5% Willow | 1% Willow | 2.5% Willow | 5% Willow | 10% Willow | 5% SC |
|---|---|---|---|---|---|---|
| **pH** | 4.87 | 4.58 | 4.33 | 4.04 | 3.75 | 5.74 |
| | **10% SC** | **0.5% Buckwheat** | **1% Buckwheat** | **2.5% Buckwheat** | **5% Buckwheat** | **10% Buckwheat** |
| **pH** | 5.21 | 5.51 | 5.37 | 5.01 | 4.66 | 4.58 |

**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

### 5.3.2 Procedures

Seeds of pea were used as the objects, moreover, this part had two independent variables: 1) sources of biomass for HTCPL i.e., seafood compost, willow and buckwheat; 2) different concentrations of HTCPL i.e., 0%, 0.5%, 1%, 2.5%, 5% and 10%. This experiment set up a blank group (water alone, 0% HTCPL), a control group (Pro-Mix $BX^{TM}$, Premier Horticulture Inc., Quakertown, Pennsylvania, USA), and twelve treatment groups. Twelve treatment group include 0.5% willow HTCPL, 1% willow HTCPL, 2.5% willow HTCPL, 5% willow HTCPL, 10% willow HTCPL, 5% seafood compost (SC) HTCPL, 10% SC HTCPL, 0.5% buckwheat HTCPL, 1% buckwheat HTCPL, 2.5% buckwheat HTCPL, 5% buckwheat HTCPL, and 10% buckwheat HTCPL. There were four replications, and each replication had 10 seeds. In terms of control group, pea seeds were randomly started in four Pro-mix-$BX^{TM}$ potting mediums contained in a 10 cell-tray. Each cell-tray had one seed. For the blank group and treatment groups, the first step was soaking rockwool cubes in the sample solutions. Each 40 Rockwool cubes were immersed in the same sample solution. After transferring the soaked rockwool cubes to trays, pea seeds were randomly sowed in rockwool cubes and each rockwool cube had one seed. A growth chamber (Conviron, Winnipeg, Canada) could provide the appropriate growing conditions including temperature (20ºC), light (330-440 μmol $m^{-2}$ $s^{-1}$) for pea baby greens growth (Murphy et al., 2010; Murphy & Pill, 2010; Brazaitytė, et al. 2015). Therefore, after seedlings coming, all the pea plants were put in a growth chamber.

*The measurement of growth parameter*

This study started recording the plant heights (stem length) 14 days before the baby greens were harvested. The plant heights were measured with a 30-cm ruler every day to determine stem growth rate as $\frac{H_i - H_1}{t}$, where $H_i$ = $i^{th}$ height measurement, $H_1$ = first height measurement, and t = the numbers of days between $H_i$ and $H_1$. The stem diameters were measured with a digital caliper on the day of harvest (Mastercraft, Michigan, USA). Selected a position where the stem is 2 cm from the root and measure its diameters. Leaf chlorophyll was measured by using a CCM-200plus SPAD meter on the day of harvest (Opti-Sciences, Hudson, USA). Selected three true leaves for each pea baby greens samples to measure chlorophyll. The true leaf number was counted on the day of harvest. The fresh weight was measured on the day of harvest.

*Analysis of mineral elements content*

After obtaining the fresh weight of the pea baby greens, all pea baby greens were placed in an ice container and sent to the PEI Analytical Laboratories.

Nitrogen analysis was done by the combustion Analysis. Weighed and analyzed dried and ground samples on a CN-2000 combustion analyzer (LECO Corporation, St. Joseph, USA) Measured the content of nitrogen from pea baby greens by thermal conductivity detection after releasing nitrogen by combustion at high temperature.

Ignited dried ground pea baby greens samples in a furnace at 550ºC to oxidize all of the organic matter. The remaining ash was dissolved in hydrochloric acid and heated. The extracts were analyzed for phosphorus, potassium, calcium, magnesium, boron, copper, zinc on an Inductively Coupled Argon Plasma Optical Emission Spectrometer (5800 ICP-OES, Agilent Technologies, Santa Clara, USA).

5.3.3 Statistic Analysis

Data of growth parameters were subjected to two-way analyses of variance (ANOVA) using Minitab version 18.1 (Minitab Inc., State College, PA, USA). Tukey's method was used to separate treatment means when the ANOVA indicated a significant difference at $P \leq 0.05$. In terms of mineral content, the variance-covariance structure of a set of variables was explained by the two-dimensional principal component analysis (PCA) using the XLSTATS premium version (Addinsoft Inc, Paris, France). Microsoft Excel was used to plot graphs.

## 5.4. Results

In terms of the growth of pea baby greens, HTCPL at appropriate concentrations has the potential to be used as a fertilizer to promote baby greens development (Table 5.1, Table 5.3 & Figure 5.1). However, this study did not find that the effect of HTCPL on the mineral content of baby greens under specific concentration and source conditions has a high overlap with the control (Table 5.4 & Figure 5.2).

Table 5.3 P-value of growth parameters for pea (*Pisum sativum* L.) baby-greens in all treatments.

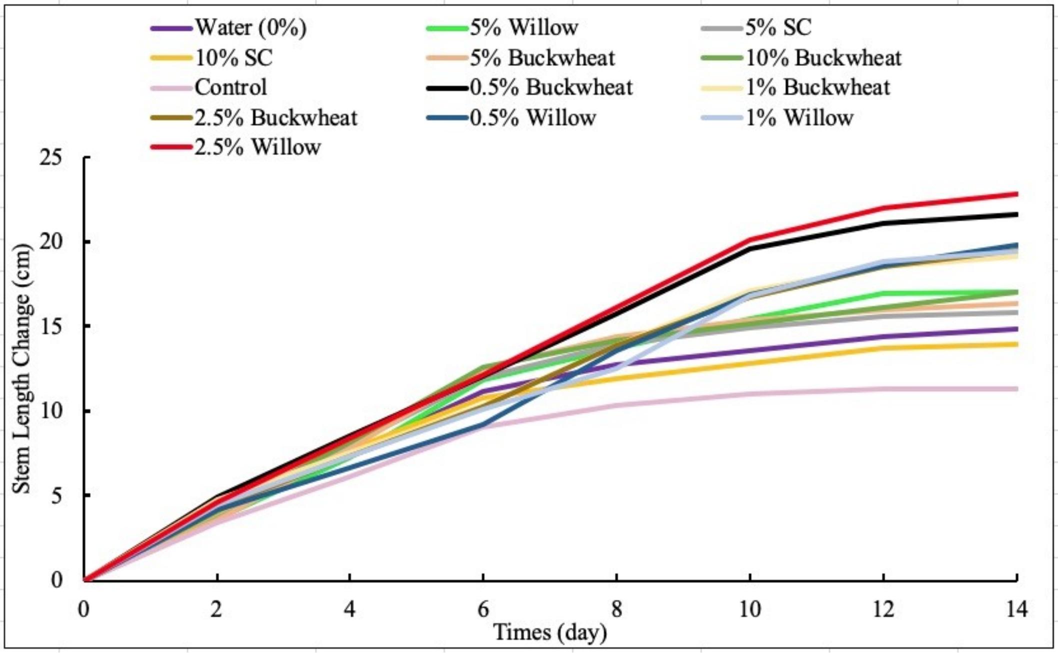
| | **Stem Diameter** | **Emergence Rate** | **Chlorophyll Content** | **Leaves Number** | **Fresh Weight** | **Plant Height** |
|---|---|---|---|---|---|---|
| **P-value** | 0.531 | 0.000 | 0.531 | 0.583 | 0.000 | 0.063 |

Table 5.4 The emergence rate and fresh weight of pea (*Pisum sativum* L.) baby-greens.

| | **Emergence Rate (%)** | **Fresh Weight (g)** |
|---|---|---|
| **Water (0%)** | 80.0 abc | 2.93 de |
| **0.5% Willow HTCPL** | 87.5 a | 4.45 ab |
| **1% Willow HTCPL** | 87.5 a | 4.27 abc |
| **2.5% Willow HTCPL** | 82.5 abc | 4.19 abc |
| **5% Willow HTCPL** | 47.5 d | 2.28 e |
| **5% SC HTCPL** | 55.0 d | 3.50 cd |
| **10% SC HTCPL** | 70.0 abcd | 3.89 bc |
| **0.5% Buckwheat HTCPL** | 85.0 ab | 4.04 bc |
| **1% Buckwheat HTCPL** | 85.0 ab | 4.50 ab |
| **2.5% Buckwheat HTCPL** | 90.0 a | 4.97 a |
| **5% Buckwheat HTCPL** | 47.5 d | 2.84 de |
| **10% Buckwheat HTCPL** | 57.5 bcd | 3.38 cd |
| **Control** | 92.5 a | 4.67 ab |

**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Pea seeds treated with the 10% willow HTCPL could not germinate. Besides the 10% willow HTCPL, only emergence rate and fresh weight had significant differences among other treatments (Table 5.3). The effect of the 0.5% willow HTCPL and 0.5% buckwheat HTCPL on pea baby greens was similar to that of the control (Table 5.3 and Table 5.4). The fresh weight of pea baby greens treated with the 0.5% willow HTCPL and 0.5% buckwheat HTCPL were significantly higher than that of the treatment with water.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 5.1 The leaves length change of pea (*Pisum sativum* L.) baby greens with different HTCPL (hydrothermal carbonization processed liquid) samples.

It is obvious that the stem growth rate of pea baby greens treated with 2.5% willow HTCPL and 0.5% buckwheat HTCPL was higher than pea baby greens treated with the other treatments (Figure 5.1). Starting from the third day of the measurement, the difference in the growth rate of stems among the treatments increased, and the growth rate of stems in the control was the lowest (Figure 5.2). There was no significant different in plant height of pea baby greens with different treatments (Table 5.1), which indicated that the growth rate of the control was higher than other treatments in the early growth of baby greens.

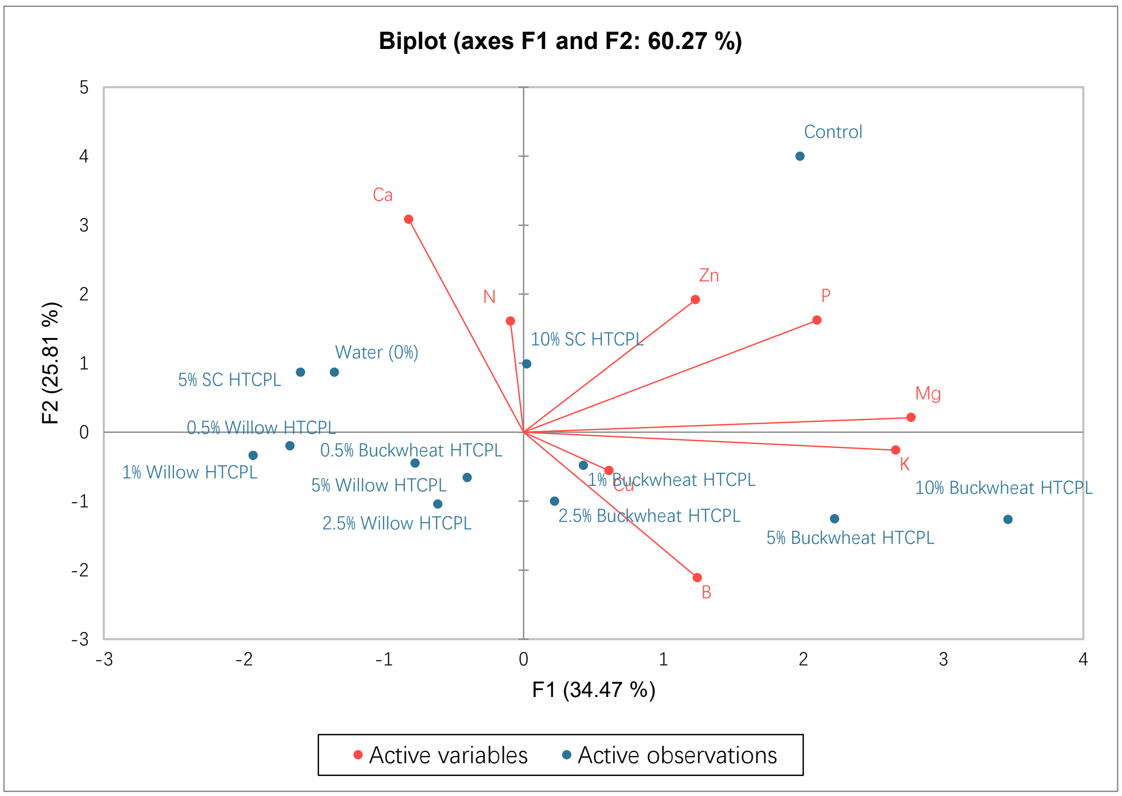
Table 5.5 The mineral analysis of pea (*Pisum sativum* L.) baby greens treated with different treatments

| Element (mg/kg) | Water (0% HTCPL) | 0.5% Willow HTCPL | 1% Willow HTCPL | 2.5% Willow HTCPL | 5% Willow HTCPL | Control | 5% SC HTCPL |
|---|---|---|---|---|---|---|---|
| **Nitrogen** | 84200 | 79300 | 78000 | 77400 | 77800 | 84000 | 90100 |
| **Phosphorus** | 6400 | 7000 | 6900 | 6900 | 6500 | 8500 | 6500 |
| **Potassium** | 21900 | 23100 | 20500 | 22700 | 24900 | 27500 | 23400 |
| **Calcium** | 3500 | 3000 | 3300 | 3000 | 3100 | 4600 | 3600 |
| **Magnesium** | 1900 | 1800 | 1800 | 2000 | 1900 | 2300 | 1800 |
| **Boron** | 16 | 13.4 | 14.6 | 16.4 | 17.8 | 12 | 17.4 |
| **Copper** | 11.93 | 12.09 | 13.51 | 13.59 | 12.53 | 12.4 | 10.95 |
| **Zinc** | 97.9 | 72.9 | 75 | 74.8 | 91.9 | 100.3 | 87.2 |

| Element (mg/kg) | 10% SC HTCPL | 0.5% Buckwheat HTCPL | 1% Buckwheat HTCPL | 2.5% Buckwheat HTCPL | 5% Buckwheat HTCPL | 10% Buckwheat HTCPL |
|---|---|---|---|---|---|---|
| **Nitrogen** | 86500 | 77700 | 75000 | 85600 | 76700 | 82800 |
| **Phosphorus** | 7300 | 7000 | 7200 | 6800 | 7400 | 7600 |
| **Potassium** | 24400 | 24000 | 26900 | 27600 | 28800 | 29400 |
| **Calcium** | 3700 | 3200 | 3300 | 2700 | 2300 | 2200 |
| **Magnesium** | 1900 | 2000 | 2100 | 2000 | 2200 | 2400 |
| **Boron** | 18.1 | 15.9 | 16.5 | 18.1 | 19 | 20.8 |
| **Copper** | 12.88 | 11.44 | 11.72 | 11.83 | 12.17 | 13.27 |
| **Zinc** | 92.4 | 72.9 | 76.2 | 74.2 | 90.4 | 90.3 |

**The results reported on a dry matter basis*
**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**HTCPL, hydrothermal carbonization processed liquid*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

There were no germinated seeds in treatment 10% willow HTCPL. Based on Table 5, The content of phosphorus, calcium and zinc in the control group was the highest, while that of boron was the lowest. The content of magnesium and copper was not much different among the treatments (Table 5.5). It was observed that regardless of the source of HTCPL, higher concentrations of HTCPL (5% and 10%) contained more zinc than lower concentrations (0.5%, 1% and 2.5%). Compared to the other HTCPL treatments, HTCPLs extracted from SC made pea baby greens have more calcium content, and HTCPL obtained from buckwheat makes pea baby greens have more potassium content.




**Buckwheat, buckwheat (Fagopyrum esculentum) green foliage*
**SC, seafood compost*
**Willow, willow (Salix babylonica) branches*

Figure 5.2 The PCA analysis showed the effect of HTCPL (hydrothermal carbonization processed liquid) with different feedstocks and concentrations on the mineral contents of pea baby greens.

The output from the PCA analysis indicated that pea baby greens treated with HTCPLs were segregated into distinct quadrants of the biplot based on the feedstocks and concentrations of HTCPL (Figure 5.2). This segregation accounted for 60.28% of the total variability observed in the data. The control had positive relationships with zinc, phosphorus, and magnesium, but the control had negative relationships with calcium and nitrogen. Compare with other treatments, the 1% and 2.5% buckwheat HTCPLs were close to copper. It was obvious that the 10% SC HTCPL was on the axes F1 in the biplot, indicating that the observation of F1 on pea baby greens treated with the 10% SC HTCPL was almost 0.

**5.5 Discussion**

In this study, there were no germinated seeds in the treatment of the 10% willow HTCPL. This is because the pH value in the 10% willow HTCPL was low (3.75) (Table 5.3), which is significantly lower than the range of pH (5.5-7) suitable for germination of most seeds (Liu & Hanlon, 2015). The composition and properties of willow as feedstock and extracted HTCPL can be mainly attributed to high acetic acid and carboxylic acids (Hager et al., 2020). This explains why willow HTCPL at the same concentration has a lower pH value than SC HTCPL and buckwheat HTCPL.

According to the measured plant growth parameters, high concentration (5% and 10%) of HTCPLs were not suitable for the growth of pea baby greens because the emergence rate and fresh weight of the treated plants were significantly lower than those of the control (Table 5.3 & Table 5.4). This may also be attributed to the low pH of the high concentration HTCPLs (Liu & Hanlon, 2015). Regardless of the raw material, the effects of the low concentration (0.5%, 1%, 2.5%) of HTCPLs and the control on the growth of pea baby greens were generally similar (Table 5.3 & Table 5.4). The small amount of nutrients present in the control was sufficient for the growth and development of baby greens. Compared with pea baby greens grown only with water, low concentrations of HTCPLs could significantly increase the fresh weight (yield) of baby greens (Table 5.3 & Table 5.4). This may be due to the low concentration of HTCPLs containing substances that affect the growth of baby greens, such as nitrogen, potassium, magnesium, phosphorus, iron, and calcium, and the pH which was closer to the optimal range for plant growth. In short, this study showed that HTCPL obtained from different biomass with the appropriate concentration has the potential to work as a bio-fertilizer for growing pea baby greens.

Baby greens like common green leafy vegetables, have insufficient protein and carbohydrate content to meet human needs but they are an excellent source of most minerals and vitamins (Altundag et al., 2011; Grusak & DellaPenna, 1999). The intake of minerals is important for humans because many minerals, such as nitrogen, phosphorus, potassium, calcium and magnesium, participate in the metabolism of protein, lipids and carbohydrates in the human body, regulate osmotic pressure and acid-base balance, and affect the development of cellar and skeletal structure (Chekri et al., 2012). For example, potassium-rich foods can be used to treat rheumatoid arthritis (Borah et al., 2009). With reference to previous studies, it is found that the contents of potassium, phosphorus, copper and magnesium in pea baby greens were close to the average level of previous studies on the mineral contents of pea baby greens (Santos et al., 2014a; Santos et al., 2014b). Magnesium plays many roles in the human body. Magnesium can participate in a variety of enzyme-related reactions, activate a variety of enzyme bodies, protect human blood vessels and heart health, and prevent heart attacks (Schachter, 1996). Previous studies suggested that the magnesium content in pea baby greens is lower than other common baby greens, and the results of this study again confirmed this view (Santos et al., 2014a; Santos et al., 2014b). It should be noted that the calcium content in this experiment is significantly lower than that in previous studies (Santos et al., 2014a; Santos et al., 2014b). This result can be due to a variety of factors including temperature conditions, light conditions, fertilizer conditions, crop genetic factors, and/or crop maturity at harvest (Grusak & DellaPenna, 1999; Sanchez-Castillo et al., 1998). The mineral contents in pea baby greens treated with 14 treatments showed that nitrogen was the most abundant macro-element present, between 75 g/Kg (1% buckwheat HTCPL) and 86.5 mg/Kg (5% SC HTCPL) (Table 5.5). Nitrogen content in plants is closely related to protein synthesis. The molecular component of proteins is amino acids (Sosulski & Imafidon, 1990). And the basic component of amino acids is nitrogen (Sosulski & Imafidon, 1990). Therefore, nitrogen content in plants is closely related to protein synthesis. Pea baby greens samples with higher nitrogen content contain more plant protein. Boron is not defined as an essential nutrient for humans, and a high intake of boron has a risk of damage to the human body (Bolt et al., 2020). Under normal conditions, the content of boron in vegetables generally does not exceed 25ppm (Berger & Truog, 1939). It is obvious that the boron content in all pea baby greens samples in this study was within the standard range (Table 5.5).

In short, from previous learning, the mineral contents of all pea baby greens treated with HTCPLs in this study were within a reasonable range and basically meets the mineral nutritional value required by baby greens (Santos et al., 2014a; Santos et al., 2014b).

**5.6 Conclusion**

This work found that HTCPL with different concentrates and feedstocks caused different effects on pea baby greens growth. It is proved that both willow HTCPL and buckwheat HTCPL has the potential to work as bio-fertilizer for growing pea baby greens at appropriate concentrations. HTCPL did not show a significant positive or negative effect on increasing the mineral contents of pea baby greens. The mineral contents of peas baby greens treated with HTCPL samples reached the standard range of mineral contents of baby greens.

# Chapter 6 Overall Conclusion and Recommendations

It was generally believed that HTCPL (hydrothermal carbonization processed liquid) was a by-product of the HTC process until its multifaceted potential value was discovered. HTC is a thermochemical process that converts organic materials to value-added products by heating them to between 180 ℃ and 350 ℃. In agriculture, HTCPL may be used as a bio-fertilizer, because the main components of HTCPL are similar to the macronutrients required by plants, including nitrogen, phosphorus, potassium, calcium, and magnesium. Meanwhile, HTCPL also contains a variety of micronutrients for plants, such as copper and iron. If HTCPL can be used in agricultural production, the environmental problems caused by traditional chemical fertilizers, such as soil structure degradation and erosion, will be alleviated. However, the fate of minerals in the HTC process is unclear. The feedstock, heating rate, residence time, heating time, reacting temperature, reacting pressure, and particle size can jointly or independently affect the composition and properties of HTCPL. Of all the factors affecting the composition and properties of HTCPL, feedstocks are usually the most influential. The composition and properties of HTCPL extracted under different HTC conditions may vary greatly. Therefore, the value of HTCPL in agriculture is less understood.

In chapter 3, HTCPLs were extracted from willow (*Salix babylonica*) branches, SC (seafood compost) and buckwheat (*Fagopyrum esculentum*) green foliage at 180 ºC and

200 ºC heating temperature respectively. It was observed that the fate of minerals in the feedstock during the HTC process was uncertain. For example, buckwheat HTCPLs retain most of the total nitrogen in the feedstock, while the total nitrogen in SC did not remain in HTCPL in a large amount. Although the feedstocks cause differences in the nature and composition of HTCPLs, the main components of all extracted HTCPLs are similar, including nitrogen, phosphorus, potassium, calcium, magnesium and sodium. In addition, it has been observed that HTCPLs contain small amounts of various trace elements required by plants, such as copper, potassium and nickel. It is suggested that HTCPL extracted from willow, SC and buckwheat all have the potential to be used as a bio-fertilizer. Chapter 3 also found that when the feedstock was the same, the content of phosphorus, potassium, calcium and magnesium was higher with 200 ℃ heating temperature compare with 180 ℃ heating temperature. Therefore, Chapter 3 recommended that the HTCPL extracted at 200 ℃ has more potential as a fertilizer than the HTCPL extracted at 180 ℃. Considering the pH, hardness, alkalinity, electric conductively, and toxic substances such as lead, HTCPL should be used on plants after being diluted.

In order to verify whether the HTCPL extracted from willow branches, SC and buckwheat green foliage at a heating temperature of 200 ℃ can be used on plants, chapter 4 tested the effects of different concentrations of HTCPLs on seed germination and seedling growth. Pea (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.), pac choi (*Brassica campestris*

L. *ssp.*), lettuce (*Lactuca sativa*) and kale (*Brassica oleracea* var. *sabellica*) seed were selected as objects. All HTCPLs did not show the obvious promoting effect on seed germination and seedling growth. In addition, willow HTCPLs with high concentrations (5% and 10%) had significant inhibitory effects, which may be related to the low pH value of high concentrations of HTCPLs. Chapter 4 also observed that the seed germination of kale, lettuce and pac choi were more sensitives to HTCPL than sunflower and pea. It is suggested that the size of the seed and the genetic characteristics of the seed cause the seeds to respond differently to treatments Based on the results of chapter 4, HTCPL is not recommended to promote seed germination and seedling growth.

After investigating the effect of HTCPL obtained from different biomasses on the seed germination and seedling growth, chapter 5 continued to evaluate the influence of different HTCPLs on pea baby greens growth and mineral composition. It is observed that pea baby greens applied with 5% and 10% HTCPLs had poor growth performance, especially pea seeds treated with the 10% willow HTCPL, which could not germinate. However, chapter 5 also found that the effects of 0.5% willow HTCPL and 0.5% buckwheat HTCPL on pea baby greens are similar to those of the control (Pro-Mix BX$^{TM}$), which means that HTCPL from different sources has the potential to work as a bio-fertilizer at appropriate concentrations. HTCPL did not show a significant positive effect on increasing the mineral composition of pea baby greens. The mineral nutritional value of the pea baby greens treated with HTCPL samples meets the level that baby greens should have. Chapter 5 suggested that it is possible to use the HTCPL extracted from different biomass as a bio-fertilizer for young crop growth.

The general hypothesis of this project is that application of the HTCPL extracted from seafood compost at 200 °C heating temperature can significantly improve seed germination, plant growth performance and plant mineral composition compared to the HTCPL extracted from willow branches and buckwheat green foliage. Obviously, the result of this project is different from the above hypothesis. This project proved that the composition and properties of HTCPL depend on the feedstocks and temperature of HTC, and the main factor is the feedstocks. In addition, it was found that HTCPL extracted from different biomasses has the potential to work as a bio-fertilizer to cultivate young crops at appropriate concentrations. Until now, there are few studies explicitly evaluating the effect of HTCPL on plants. Therefore, more tests will be required to confirm the results and efficacy of HTCPL prior to making recommendations to farmers.

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