VERMICOMPOSTING, WASTE RECYLING

AND PLANT GROWTH

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Vermicomposting has been proposed as a sustainable technique for managing various types of organic wastes. The aims of this study were to test the feasibility of vermicomposting two of Canterbury's problematic wastes (organic municipal waste and the used animal bedding) and to evaluate the vermicompost generated from them for use in horticultural production.

The research first examined how changes in waste proportions and types affected earthworm growth and reproduction. Vermicompost quality in terms of its nutrient and agronomic values was then considered, through the use of a series of pot experiments with Pak Choi plants. These experiments investigated influencing factors such as the type of medium, source of vermicompost, application rate, application method, processing method and chemical fertiliser addition on plant growth.

The results of the vermicomposting experiments showed that the two waste streams could be a valuable food source for earthworms, and it found that the ideal combination in terms of earthworm growth and reproduction and final vermicompost quality was a mix of 80% fresh shredded waste and 20% used animal bedding. The vermicompost produced from this mix generally had good agronomic value, but a low nitrogen content.

Coir had high vermicompost use efficiency, and plants grown in coir with a 10% vermicompost addition grew as well as plants in a standard potting mix. Mixing chemical fertiliser and vermicompost together with coir led to further improvements in plant growth, compared with the use of vermicompost alone. These results demonstrated that it is feasible to recycle the two problematic wastes by vermicomposting, and that the vermicompost produced is beneficial as a plant fertiliser. Coir could be a promising material for use in horticultural production when combined with vermicompost. However, further research is required to investigate ways of using these resources in the most efficient manner.

Keywords: Vermicomposting, Municipal organic wastes, Used animal bedding, Vermicompost quality, Plant growth, Coir.

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Thanks to everyone who I met in the year for getting into my life and giving me joy.

Thank you all for showing me the lives in different dimensions, just like stars in the darkness night encouraging me to build a willingness to create the own brightness and believing the real value of life.

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Chapter 1: Introduction

1.1 Introduction to New Zealand's waste problems and vermicomposting

Waste generation has grown to be a serious problem worldwide (Lim et al., 2014). In New Zealand, a report from the Ministry for the Environment documented that 3.156 million tonnes of waste were sent to landfill in 2006 (The Ministry for the Environment, 2007). Of all the waste types, organic waste is the largest sort of waste and costs about NZ$751 million annually to process (Yates, 2013). Despite the fact that the government has made efforts on recycling waste, many organic wastes still haven't been efficiently recycled (Christchurch City Council, 2013).Thus, there is an urgent need to improve the waste management system for meeting the requirement of the government's "zero waste strategy" in New Zealand.

Among various waste recycling methods, vermicomposting has been suggested to be one of the sustainable techniques for managing various types of organic wastes (Sim & Wu, 2010). Vermicomposting is an earthworm-involved process, in which specially selected compost earthworms digest organic matter in their gut, and produce vermicompost or cast. The final product of vermicomposting, vermicompost, is humus-like with fine sized particles and has few contaminants with desirable aesthetics (Nedgwa et al., 2000).

Vermicompost has many advantages over traditional compost in terms of its physical structure, nutritional content and biochemical value due to the higher mineralisation and humification rate through the vermicomposting process (Lim et al., 2014). Vermicomposting is considered to be a low-cost technology system for the processing of organic waste because some of the effects of earthworm activities (e. g. substrate aeration, mixing and grinding) save a lot of energy compared to the traditional microbial composting process, where these mechanical activities are done by microorganisms (Nedgwa et al., 2000). In addition, vermicomposting takes less processing time and produces greater fertiliser value with a higher humus content and less phytotoxicity compared with the traditional composting (Sim & Wu, 2010). Since New Zealand produces large amounts of organic waste every year, there would be a great saving in primary plant nutrients and metabolic energy if these organic waste types could be transformed to vermicompost and used in agriculture and horticulture. Besides, vermicomposting significantly reduces the quantity of waste and decreases the initial volume of raw materials by 84% to 89% depending on the composition of wastes, in a relatively short time (Hanc & Chadimova, 2014). Thus, it can also save costs from waste transportation and disposal (Wani et al., 2013).

1.2 Waste problems in Canterbury

The effectiveness and success of vermicomposting are largely determined by the earthworm feedstocks (Sim & Wu, 2010). In terms of waste recycling, the feeding material for earthworms is generally limited by the types of available waste. Despite earthworms having the potential to digest all types of organic materials, many are still not suitable for feeding earthworms directly without extra processing (Yadav & Garg, 2010). If the feeding materials are not appropriate for earthworms to digest, a high mortality rate is usually observed, and there may be effects on the sexual development of earthworms (Yadav & Garg, 2010; Kaushik & Garg, 2003). In addition, since the characteristics of waste material may vary depending on its source and decomposing stage, the waste decomposing rate could be significantly different even though the wastes are in the same name (Yadav & Garg, 2010). Therefore, the tests of feasibility of wastes used in vermicomposting are essential before adapting vermicomposting techniques for waste recycling in practice.

In the Canterbury region of New Zealand, two organic waste streams need to be recycled. The first waste stream is the organic municipal waste collected in green wheelie bins from local households weekly in the Selwyn District of Christchurch. This waste is a mix of all types of organic wastes generated from local families in the district with a substantial garden waste content. However, due to limitations of the composting facility, only few of the waste type can be thermophilically composted. Most of this organic waste are sent directly to landfill after windrowing. As a result of the raise in municipal waste disposal price, Selwyn District Council is looking for an alternative method to decompose of this waste. The second waste stream is used animal bedding from dairy farms in Eyrewell, Canterbury, New Zealand. The used animal bedding is made up of small wood chips mixed with dairy cow slurry. This waste has been piled outside the farms in Eyrewell and left for several years. However, the manured wood chip seems unsuitable as a feeding material for earthworms directly and a very high mortality rate was noted due to its low pH and high ammonia content (Chapter 3).

In consideration of the characteristics of the two types of wastes, it is likely that the disadvantages of them (the low pH in dairy waste and low nutrient content in the municipal waste) could be diminished by mixing them together. However, the optimal proportions and types of these waste mixes needs to be investigated. Thus, this study will partly contribute to this area of research subject by examining the changes in vermicomposting efficiency and final vermicomposting quality with different types and proportions of mixed waste.

1.3 Requirements of industrialised vermicompost production

Not only can vermicomposting be a waste recycling method, but it can be a potential way to create profits for an industry by selling vermicompost.

Since vermicompost is mainly used as an amendment to a soil or plant growth medium, the quality of vermicompost as a fertiliser is the primary need for the vermicomposting industry to consider. The quality of vermicompost, however, is not just one parameter but an overall description of various characteristics of vermicompost, including physical characteristics, chemical characteristics, biological characteristics, and plant nutrient content (Edwards et al., 2011). For the vermicomposting industry, the most important thing is to establish a secure market by providing products with a stable agronomic value. However, the relationship between the quality parameters and agronomic value of vermicompost is still not clear.

The actual effect of vermicompost on plant growth can vary significantly depending on the source of the vermicompost, application rate and cultural conditions. Studies have tested responses of many plant species to different vermicompost application rates and sources (Atiyenetal, 2001; Arancon, 2008; Am-Euras, 2009; Morales-Corts et al., 2014). Some factors such as application methods have not been investigated previously, and despite similar types of waste having been used in vermicomposting in the past, no previous research has tested the effects of vermicompost derived from the two domestic waste types in Canterbury on plant growth. For the future development of a vermicomposting industry based on recycling wastes in Canterbury, it is necessary to test the agronomic value of the vermicompost derived from these wastes and how much cultural conditions can affect its performance. As the reseasons above, this study will also investigate the correlation between the quality parameters and the agronomic value of vermicompost as well as the influencing factors on plant growth.

1.4 Research objectives

As a result of the problems and limitations described above, the objective of this study were to:

- Examine the feasibility and effectiveness of using municipal waste for vermicomposting, particularly for the wastes from Selwyn District Council.

- Investigate the effects of blending municipal waste with a dairy industrial waste on the effectiveness of vermicomposting systems.

- Investigate the effects of vermicompost as a plant substrate amendment on different growth stages of plants (*Brassica rapa* subsp. *chinensis*).

- Evaluate the effects of vermicompost derived from different sources on plant growth.

- Investigate the factors which may affect the responses of plants to vermicompost application

Chapter 2: Background and Literature Review

2.1 Background

Earthworms are known to be ecosystem engineers which help to improve soil aeration, drainage and water holding abilities (Jones et al., 1994). They play a crucial role in soil formation and fertility, not only functioning as an element of a food web but also being responsible for altering dynamics of the ecosystem through the maintenance, modification and creation of habitats for other organisms (Jouquet, 2006).

The study of earthworms was started by Charles Darwin who made the first report on the role of earthworms in the breakdown of organic matter in the ecosystem in 1881 (Lowe et al., 2014). In the early twentieth century, some more economically effective materials, which were usually animal wastes, were successfully used to grow earthworms in the United States. After the success of intensive culture of certain epigeic earthworm, Oliver (1937) and Barrett (1942) both noticed the agriculture value of earthworm casts of some epigeic species and suggested vermicast or vermicompost could be used by farmers to improve agricultural soils and crop production (Edwards et al., 2011). After this, the vermiculture industry expanded rapidly all over the world for producing earthworm bodies and vermicompost, and they were sold as fishing bait, animal feeds and plant fertiliser. With the increase of relevant studies, research on a few epigeic earthworms used in vermiculture and the process of vermicomposting has been a separate subject from general earthworm studies, due to the demand of vermiculture industry needs and the value of vermicomposting on recycling organic wastes.

Recently, the cultivation of earthworms in organic waste has progressed considerably. For example, studies of optimal growth conditions have been established and well-developed for some commonly used compost earthworm species. Many wastes such as animal wastes (Jayakumar et al., 2011; Luth et al., 2011; Ngo et al., 2013), sewage sludge and solids (Nogales et al., 2005; Pereira et al., 2014), paper industry wastes (Arancon, Edwards, Bierman, Metzger & Lucht, 2005; Quintern, 2014), plant residues (Fernández-Gómez et al., 2010; El-Haddad et al., 2014; Morales-Corts et al., 2014); human faeces (Yadav et al., 2010, 2011, 2012); food industry wastes (Adi & Noor, 2008; Hanc & Chadimova, 2014) and various types of mixed wastes were reported to be feasible to use for vermicomposting production. Furthermore, the suitability of vermicompost as a plant growth medium and it potential value in the horticulture industry has also been confirmed. Nevertheless, there are still many undeveloped areas needing to be addressed. The following literature review will briefly summarise relevant research on vermicomposting processes, vermiculture and plant responses to vermicompost additions.

2.1.1 Vermicompost and the vermicomposting process

Vermicomposting is a similar process to composting, but with the addition of certain epigeic earthworms to assist in the decomposing process. However, vermicomposting produces a distinctive end product, known as vermicompost or vermicast, and the properties of vermicompost are different to regular compost in both physical structure and biochemical composition (Edwards et al., 2011). Since vermicompost is the major product to the commercial vermicomposting industry, and most initial organic materials will eventually transform to earthworm cast after vermicomposting, research on vermiculture mainly focused on the process of vermicomposting.

In the vermicomposting process, earthworms have multiple actions on waste degradation by acting as an aerator, grinder, crusher, chemical degrader and biological stimulator, and the original characteristics of the initial materials are therefore physically and biochemically changed in a relatively short time (Sinha et al., 2010). Apart from the activities of earthworms, mesophilic microorganisms also play an important role in the conversion of organic wastes. In fact, the vermicomposting process involves a complex food web in which various types of microorganisms, invertebrates and insects may combine to result in the recycling of organic matter and the release of nutrients. In the process, many biotic interactions including competition, mutualism, predation, and facilitation may occur between microorganisms and fauna in the vermicomposting system (Sampedro & Dominguez, 2008). Therefore, as all of these activities eventually lead to the formation of vermicompost or the degradation of organic matter, it is difficult to predict the rate of decomposition in the system (the vermicomposting efficiency) without considering these interactions. On the other hand, considering all of these interactions make the study of vermicomposting extremely hard and complex. Thus, a possible compromise is to focus on a few crucial nodes (organisms) in the food web of the vermicomposting system.

The most important nodes, in the food web of the vermicomposting system, are the earthworms and microorganisms. The microorganisms such as bacteria, fungi and ciliates are the most abundant and diverse members which help to break down organic food residues (Edwards et al., 2011). Many studies have demonstrated a complex interaction between earthworms and microorganisms in vermicomposting systems (Gomez-Brandon & Dominguez, 2014). Firstly, the activities of microbes can enhance the degradation of initial materials which can, at a certain stage, help the decomposition process of earthworms. Also, the formation of vermicast indirectly stimulates microbial populations as a result of the greater surface area available for microbial colonisation. However, a competition of degraded organic matter may occur with the process of mineralisation of organic materials (Gomez-

Brandon and Dominguez, 2014). Thus, earthworms affect the structure of the microflora and microfauna communities by their activities (Figure 2.1). Although earthworms have a close relationship with microorganisms and microfauna, it still not clear how they specifically interact with each other. Research in this area is still undeveloped, and more studies are needed to fully understand the mechanisms of the vermicomposting system.

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Figure 2.1 Positive (+) and negative (−) effects of earthworms on microbiota and microfauna

*Adapted from Gomez-Brandon and Dominguez (2014).

2.1.2 History of vermiculture and commercial vermicomposting in New Zealand

In New Zealand, the use of *Eisenia foetida* to break down organic wastes started in the 1970s and reached its peak in the 1990s. During this period, vermicomposting industries moved from the limited sales of earthworms as fish bait into vermicomposting for organic waste management, influenced by the success of large-scale municipal vermicomposting facilities in the United States. In 1995, the New Zealand Earthworm Association was established with about 200 people attending the first seminar. However, the association quickly failed after a short flourish, followed by a decline of the vermicomposting industry. Edwards et al (2011) concluded in their book that this failure was due to the spread of earthworm "buy-back" schemes, which were based on pyramid-selling techniques where profits depended on attracting people to grow earthworms, which were then sold to other potential new earthworm growers at considerable profits to the entrepreneurs.

Although the "buy-back" schemes eventually failed and ceased, their influences on the vermicompost industry in New Zealand still remain. There was a big loss of key enthusiasts and large operations in the 1990s which hindered the development and maturation of vermiculture technology. A direct result of the “buy-back” schemes is that the current vermiculture industry is mainly in the form of small domestic earthworm farm based on outdoor windrowing systems. Only 82% of operations require more than two people to operate them with one-third of them spending fewer than five hours per week on their management. In addition, growing earthworms and selling them to new growers was reported to be the main focus of most vermiculturists (about 90 %) (Parliamentary Counsel Office, New Zealand, 2008). This situation is also probably due to the support from local governments because they have subsidised earthworm farms (about 8%) to encourage households to vermicompost their own food waste (Webster & Taylor, 2009).

However, the mode of small domestic earthworm farms relying on selling earthworms is not sustainable or profitable. This is because the demands of compost earthworms are relatively fixed, and it is unlikely that local households will repurchase earthworms once their own earthworm farm has been set up. Although there may still be new households and growers willing to buy earthworms, the market will be limited in the future. In addition, as a result of the low average productive efficiency of these small earthworm farms, the prices of both compost earthworms and vermicompost in New Zealand are significantly higher than in the global market. On the one hand, it provides opportunities for large-scale operations with more effective vermicomposting systems to make considerable profits. On the other hand, the high price actually hinders the spread of vermiculture products and limits the uses of vermicompost for agricultural production. Many small earthworm farm growers were just raising earthworms based on their own experience. They usually lack knowledge of earthworm physiology, taxonomy, ecology and quality management. This leads to many problems such as the mixing of earthworm species and misguidance to new growers so that the health of earthworms and the quality of the vermicompost cannot be guaranteed.

Edwards et al (2011) proposed that vermicomposting facilities must focus on waste management and marketing vermicompost in order to achieve long-term success, after reviewing the development of vermiculture in US and other countries. He recommended that on-site vermicomposting should be kept to a limited scale considering the cost of transport and possible joint partnership with nearby facilities. This could also be a possible direction for the further development of the New Zealand vermiculture industry. A large-scale vermicomposting site based on composting pulp mill solids and sewage sludge has been successfully established and run in Tokoroa, Waikato, New Zealand. (Quintern, 2014). Thus, the development of a New Zealand vermicomposting industry based on waste management and the supply of products to the agricultural market still looks promising despite the remains of some setbacks.

2.2 Importance of raw materials

In order to effectively use vermicomposting in waste management, several factors which may influence the growth and reproductive potential of earthworms need to be considered.

One crucial factor is the property of raw materials (feeding materials). Manna et al. (1997) pointed out that “a favourable feeding environment is a prerequisite for the success of vermicomposting in outdoor culture beds”. Because of this, many studies have further worked on discovering how environmental factors and the characteristics of raw materials can affect earthworms. The factors, as summarised by Yadav and Garg (2011), can be divided into two groups, abiotic factors and biotic factors. Abiotic factors include moisture content, pH, temperature, aeration, feed quantity, light and C: N ratio. Many studies worked on discovering the optimum conditions for earthworm growth and have found a range of optimal abiotic conditions for earthworms (Table 2.1). Biotic factors such as stocking density, microorganisms and enzymes are also important, but are more complex and variable than abiotic factors, depending on the earthworm species and the specific situation of the vermicomposting process. However, these factors are not separate, and interact with each other. For example, a vermicomposting system with excessive moisture content can cause poor aeration. The oxygen level of a substrate also relates to temperature, structure and composition of the initial materials (Yadav & Garg, 2011). Furthermore, it is noticeable that these abiotic and biotic factors are all either directly or indirectly related to the property of the raw materials (feeding materials). Thus, it is hard to evaluate the feasibility of a material used in vermicomposting purely based on its characteristic parameters without actual testing it, especially when the feeding materials interact with earthworm species and variable environmental conditions. However, there is no doubt that raw materials play an important role in the vermicomposting process with influences on the growth and reproduction of earthworms, the vermicomposting efficiency, and the final quality of the vermicompost produced.

Table 2.1 Optimum range of abiotic factors affecting the vermicomposting process

Abiotic Factors	Optimum Conditions
Moisture Content	60%-80%
Temperature	12-28°C
pH	5.5-8.5
Aeration	Adequate (turning may be required)
Light	Dark (Keep away from light)
Feeding Quantity	100 -300mg/g body weight/day
C:N Ratio	20:1-25:1

* Sourced from Yadav and Garg (2011).

2.2.1 Effects on earthworms

Since the raw materials are the main food source for earthworms in a vermicomposting system, the quality and quantity of these initial materials can directly influence earthworm growth and activity.

If the raw materials are not appropriate for earthworms, high mortality usually occurs with a slow composting rate under severe conditions. For example, Yadav and Garg (2010) reported that the earthworm, *Eisenia foetida,* was unable to survive in 100% of plant sludge from the food industry. Katheem et al. (2015) also documented that earthworms cannot survive in fresh cattle solids, pig solids and fruit and vegetable waste without bedding materials.

Generally, deaths were considered due to an unsuitable environment and a high content of harmful substances in the raw materials (Suthar 2008). Unsuitable environmental conditions such as an extremely high or low pH, excessive moisture content and poor aeration normally result from the inherent characteristics of the raw materials. For instance, a high level of ammonia, salt, polyphenols, inert materials, heavy metals, glass, plastics, pharmaceuticals and detergents were reported toxic to earthworms (Yadav & Garg, 2010). Sinha et al. (2010) found that earthworms are sensitive to the salt content in feeding materials, and the salt tolerance of earthworms depends on their species. Gunadi and Edwards (2003) demonstrated that feed materials with high electrical conductivity, high NH_4 content, high moisture content or low pH can be fatal for *Eisenia foetida*.

Nevertheless, despite many studies having mentioned some toxic substances for earthworms, there is a lack of specific research to extensively test these substances, and the mechanisms of how they affect earthworm remains unknown. Thus, further research may be required to fill these gaps.

Growth and reproductive rate of earthworms can be affected by raw materials. Kaushik and Garg (2003) observed inhibition of growth and sexual development for *Eisenia foetida,* and the nutrient content of the final vermicompost declined when the proportion of textile mills sludge was over 30% of feeding materials. However, these negative effects can be diminished by mixing with other materials.

Many studies have tested the feasibility of mixing various wastes in different proportions. Adi and Noor (2009) found that cow dung mixed with coffee grounds (30:70) showed the highest reproduction activity with high production of new earthworms, compared to raw materials made of cow dung/kitchen waste (30:70) and cow dung/kitchen waste/coffee grounds (30:35:35). An experiment by Fernandez-Gomez et al. (2010) showed an increase of earthworm growth and reproductive rate when damaged tomatoes were mixed with straw (in a ratio of 2:1 and 4:1) as a feeding substrate. In contrast, significant decreases in cocoon production were observed when cow dung was mixed with the damaged tomato waste in the same ratio (2:1 and 4:1).

According to these studies, it seems that the structure and nutrient content of raw materials are crucial for earthworm growth and development when environmental stresses are not fatal for the worms. Suthar (2006) found that the growth and reproductive rate of the earthworm *Perionyx sansibaricus* were consistently related to the initial N content of different substrates, but there were no clear effects of carbon/nitrogen (C: N) ratio on earthworm growth and reproduction. However, a contradictive result was found by the same author that cocoon production rate of the earthworm *Eudrilus eugeniae Kinberg* did not show an obvious correlation with N content, but C: N ratio showed a clear negative relation with reproductive rate (R2 =- 0.72, P<0.01) (Suthar, 2008).

In addition, Sangwan et al. (2008) recorded that a 10% horse dung and 90% sugar mill filter cake mixture was the best feed composition for both earthworm growth and reproduction. Nevertheless, the best feeding mixture was neither the best on nutrient content nor C: N ratio. In fact, the C: N ratio (34.9) of the mixture (10% horse dung and 90% sugar mill filter cake) was outside the optimum C: N range for earthworms (20:1-25:1). However, the average net weight gain (900mg) and cocoon production rate (0.51, number of cocoons produced/earthworm/day) in the optimum treatment was significantly higher than the treatment of a 50: 50 horse dung and sugar mill filter cake mix (410mg and 0.16) despite the fact that the C:N ratio of this treatment was in the optimum range (22.5). Thus, both the C: N ratio and the N content of raw materials should not be an absolute indicator for earthworm growth and reproduction without considering other influencing factors.

These results also indicated that there may be a complex interaction between chemical composition, nutrient content and physical structure of raw materials. The form of carbon and nitrogen content along with the physical structure of the raw materials is probably more important than the total content of the various nutrients. Nevertheless, there has been very little research on digestibility and the influence of different nutrient forms for earthworms. This could be a promising research direction for future studies.

Not only is the quality of the raw material crucial for the growth and reproduction of earthworms, the quantity is also important. Reductions of earthworm biomass and cocoon production rate were observed when feeding materials were in short supply. In an extreme condition, earthworms can die due to the lack of a food source. Gunadi and Edwards (2003) reported that most *Eisenia foetida* could not survive without a new substrate addition for 60 weeks (8 earthworms in 100g various wastes with a moisture content of 80-85%). An increased production of cocoons was also observed followed the addition of new substrate at the second week of their vermicomposting experiment.

These results indicated that adequate amounts of feeding materials are necessary for maintaining earthworm growth and reproduction. Adding new feeding materials into the vermicomposting system could be a way to stimulate earthworm growth and reproduction when the food source is not sufficient.

2.2.2 Influences on vermicomposting efficiency

Apart from influencing earthworm activities, the characteristics of the raw material partly determine the decomposing rate of the raw materials.

Different materials naturally have different degradation rates. For example, cellulose requires a longer decomposing time than proteins and carbohydrates (Warman and Anglopez, 2010). Generally, the easier a material can be degraded by microorganisms and earthworms, the quicker the decomposition rate (higher vermicomposting efficiency) can be achieved. Nedgwa et al. (2000) reported that the maximum microbial decomposition rate occurred when the C: N ratio was about 25. Nevertheless, the vermicomposting efficiency for a particular type of waste is not fixed, but can be greatly changed by adding amendments. For instance, lime, zeolite and various enzymes were reported to have a positive effect on the vermicomposting process (Edwards et al., 2011). Singh et al.(2013) found that inoculations of *Trichoderma harzianum*(ATCC PTA 3701), *Pseudomonas monteilii* (HQ995498), *Bacillus megaterium*(ATCC 14581) and *Azotobacter chroococcum* (MTCC 446) together in distillation waste significantly enhanced the degradation of cellulose (58.44%), hemicelluloses (29.44%) and lignin (65.23%) as well as increasing vermicomposting efficiency and final yield of the vermicompost by 15%. The increased vermicomposting yield was probably due to the faster decomposing time and that less nutrient was going to the earthworm mass since earthworms require time to complete their multiplication. Nedgwa et al. (2000) reported that earthworms can selectively utilise microorganisms as their food source.

As vermicomposting is a continuous process, the composition of raw materials is constantly changing in a vermicomposting system due to earthworm and microorganism activities. Thus, how to maintain the optimal growth conditions for the earthworms and maximise the vermicomposting rate are crucial for the success of the vermicomposting industry. Gunadi and Edwards (2003) emphasised the importance of maintaining a continuous culture of earthworms, especially when vermicomposting forms part of the waste management system. However, most of the studies were based on enclosed vermicomposting systems where the feeding substrate was added once in the system before the experiment was conducted (Suthar, 2007; Warman & Anglopez, 2010; Garg & Gupta, 2011; Sahariah et al., 2015). The choice may be partly due to the unknown feeding rate for particular raw materials and earthworm species. It is still not know how different the culture systems (continuous culture and enclosed culture) affect earthworm growth and vermicompost quality. Therefore, further research is needed in this area.

2.2.3 Correlation with vermicompost quality

The characteristics of the initial materials can also influence vermicompost properties. For example, Warman and Anglopez (2010) observed that vermicompost produced from kitchen waste /paper waste was finer and darker than vermicomposts from kitchen waste/yard waste and cattle manure/yard wastes (at a ratio of 5:1 for all waste mixture). They also found that the initial material was still traceable in earthworm cast under a microscope after vermicomposting for 90 days, and pointed that the vermicast could be an alternative food source for earthworms.

Research has shown that there is a strong correlation between vermicompost quality and initial material. The quality of vermicompost involves with various physical, chemical and biological parameters such as solids composition, bulk density, moisture content, acidity, plant nutrient content, enzymatic activity and microbial community composition (Edwards et al., 2011). Depending on the source of the raw materials, the differences in these characteristics can be quite distinct. For example, El-Haddad et al. (2014) found that the bulk density of vermicompost derived from pure rice straw (190.3 Kg/m^3) was significantly lower than the treatments supplemented with cattle dung (C/N ratio at 30), which were generally over 600 Kg/m^3. Also, there was an improvement (2.4%) in the N content of vermicompost obtained when rice straw was amended with inorganic K and P (K_2O and P_2O_5), compared to the control with pure rice straw (0.82%). Similarly, Pramanik et al. (2007) reported that the application of lime (5g/kg) to various organic wastes (cow dung, grass, aquatic weeds, and municipal solid waste) increased the nutrient content of the vermicompost, especially when it was combined with microorganism inoculation (*Bacillus polymyxa*). These results indicated that not only can amendments added in vermicompost affect vermicomposting efficiency, but they can also influence the characteristics of the final vermicompost (quality parameters).

Many studies have found that vermicomposts derived from different wastes have dissimilar autochthonous microbial communities (Fernandez-Gomez et al., 2011). The functional diversity of these vermicompost microbiota is still unclear despite inoculation of a few types of microorganisms had shown positive effects on nutrient content in vermicompost. Additionally, during the vermicomposting process, earthworms can accumulate metals in their intestines by fixing metal ions into a form of organo-metallic complex (Song et al., 2014; Sahariah et al., 2015). A reduction in human pathogens was also reported in several articles. Monroy et al. (2009) reported that the coliform population in pig slurry was reduced by 98% after being processed in earthworms' guts. Studies by Brown and Mitchell (1981), who tested the effects of *Eisenia foetida* on *Salmonella* populations, also found that *Salmonella* populations were decreased by 97.8% to 99.9% in a culture in the presence of *Eisenia foetida,* compared with cultures without earthworms (Edwards et al., 2011). Tittarelli et al (2007) concluded that hazardous heavy metals and pathogenic microorganisms will be less problematic in vermicomposts than in conventional composts, and Edwards et al. (2011) argued that vermicompost should meet the same health standard for thermophilic compost in terms of human pathogen content.

Since the characteristics of vermicomposts can be greatly affected by the initial materials, it is hard to ensure the quality or potential agronomic value of a vermicompost. Therefore, quality criteria of vermicomposts are needed, especially when composting and vermicomposting are progressed simultaneously.

If a product can be called as vermicompost, it must come from organic wastes which are predominantly broken down by earthworms. However, in practice, vermicomposts are commonly mixed with thermophlic composts and then sold as vermicompost. Edwards et al. (2011) proposed three methods to assess whether a process is vermicomposting by 1) monitoring the temperature of the processing material (temperature should lower than 45°C); 2) evaluating the aerobicity of the processing material; 3) calculating the density of earthworms in the material being processed.

In terms of the quality of a vermicompost, there are no fixed criteria as the preferable characteristics largely depend on its intended use. On the other hand, vermicompost quality is actually very hard to control since a complex food web is involved in the vermicomposting system. A practical method is to characterise vermicompost quality under an average population density for a certain earthworm species (Edwards et al., 2011). Also, since vermicompost is commonly used as a soil/ substrate amendment for plant growing, the nutrient requirements of plants also need to be considered along with the nutrient content and quantity of the vermicompost. Some crucial quality criteria of vermicomposts and plant requirements is summarised from the works of Edwards et al. (2011) and listed in Table 2.2.

Table 2.2 Quality criteria for vermicompost and the nutrient requirements of plants

Characteristics	Standard
Organic Matter Content	Greater than 20%-25%
Moisture Content	30%-50%
Maximum Particle Size	Less than 0.2mm
Inert Materials	Less than 0.5%-1% by weight
pH	6-7
Air Space	20%-30% for potting substrate
Cation-Exchange Capacity	50-100 meq/L
Soluble salt content	Not exceeds 1-2dS/m for seedlings; 2-3dS/m for established plants
Total Nitrogen	More than 100mg/L in potting medium
Total Phosphorus	0.1% for seedlings and sensitive plants; 0.5% for established plants
NH_4^+-N	Less than 10% of total nitrogen (N)

* Sourced from Edwards et al. (2011).

2.3 Vermicompost application on plant growth

Since vermicomposts are rich in plant nutrients, many studies have tested their nutrient value of by evaluating their potential commercial value as a soil/substrate amendment for horticultural production.

Most published research observed positive influences on plant growth parameters with various amounts of vermicompost applied to crops such as maize (Souza et al., 2013), lettuce (Ali et al., 2007), potato (Bhattacharya et al., 2012), pepper (Arancon, Edwards, Bierman, Metzger & Lucht, 2005; Bachman & Metzger, 2008), tomato (Atiyeh et al., 2000; Atiyeh et al.,2001; Arancon, Edwards, Bierman, Metzger, Lee & Metzger, 2003; Zaller, 2007; Bachman & Metzger, 2008; Doan et al., 2013; Olivares et al., 2015) and strawberry (Arancon, Edwards, Bierman, Metzger, Lee & Welch, 2003; Arancon et al., 2006; Singh et al., 2010).

However, an addition of vermicompost did not always show positive effects on plant growth depending on plant species, maturation and the volume of vermicompost used (Am-Euras, 2009). For example, a poor seed germination rate of Petunias was observed when seeds were sown in a commercial potting substrate (MM360) consisting of 80%, 90% and 100% cattle manure vermicompost (Arancon et al., 2008). Atiyeh et al. (2001) found that tomato seedlings grown in 100% pig manure vermicompost resulted in significantly lower weight, fewer leaves and shorter plant height than the control of 100% MM360 (a commercial soilless substrate, Metro-mix 360). They also found that substitution of MM360 by 30-40 % vermicompost had the greatest promoting effects on vegetative growth, but that 5% vermicompost resulted in the most significant increase in tomato seedling growth. Edwards et al. (2011) concluded that vermicompost added at as low as a rate of 5% was enough to make substantive increases in plant growth. Most research found that the optimal vermicompost concentration was about 20%-40% (Atiyeh et al., 2001; Arancon et al., 2008; Morales-Corts et al., 2014). However, Wilson and Carlile (1989) reported the best growth of tomatoes, lettuce, and peppers were found at application rates of 10%, 8% and 6% respectively in peat medium using duck waste vermicompost. Scott (1988) observed difference in optimum application rates (various from 20% to 50%) of vermicompost derived from various sources (cattle manure, big manure and duck waste) in a sand and peat medium.

These variations in optimal vermicompost application rates suggest that plant species, medium, and source of vermicompost can greatly influence the effects of vermicompost on plant growth. Therefore, the previous studies cannot be compared directly as plant species, source of vermicompost and medium used were various. There is a shortage of studies to extensively test how much these factors (plant species, medium, and sources of vermicompost) can affect the performance of vermicompost on plant growth and whether there are interactions between factors. Application method may also affect vermicompost use efficiency.

The importance of placement of chemical fertiliser (e. g. broadcasting and banding) has been noted especially for field horticultural production (Fukuda et al., 2012). However, there is no published information on the differences between application methods for vermicompost, although a few studies have tested the effects of vermicompost tea on plant growth using a foliar spray/application (Al-Dahmani et al., 2003; Scheuerell & Mahaffee, 2006; Haggag & Saber, 2007; Pant et al., 2009). One study reported that surface application of vermicompost derived from grape residue under straw mulch had a greater effect on the growth of grape vines compared to vermicompost applied to an uncovered soil surface and suggested vermicompost may degrade when exposure to sun and air (Magdoff & Weil, 2004). Thus, it is likely that the influence of vermicompost application method on plant growth and development are different from those of chemical fertiliser or traditional compost, and future research may be needed in this area.

2.3.1 Effects on plant growth and development

Vermicompost has been shown to have effects on the growth, yield, and quality of plants at various growth stages, with extensive influences on various vegetative parameters.

Many studies have found many positive effects after vermicompost application including enhanced growth parameters and increased nutrient contents of plant tissues. These results from previous research have been summarised by Joshi et al. (2015) and are listed in Table 2.3.

As can be seen from the table, similar results has been found by several authors that vermicompost has effects on stimulating seed germination (germination percentage/rate and germination time), enhancing vegetative growth and plant structure (plant height, shoot length, root length, number of leaves, leaf area, shoot weight, root weight, stem diameter, number of branches), increasing reproductive growth (flower head diameter, size of flower, number of flowers, length of inflorescence, fruit length, fruit size, fruit yield, number of fruit per plant, seed number, seed weight), improving quality value (content of N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, B, protein, fat, phenol, carbohydrate, dietary fiber, chlorophyll a, b, carotenoids) and economic production (seed yield, oil yield, marketable yield). In addition, it is noticeable in some experiments that the growth parameters of plants under vermicompost treatment are increased over control with chemical fertiliser in optimal conditions. For example, Uma and Malathi (2009) found the average height and number of branches of *Amaranthus* were increased with vermicompost application on the 30 days after germination. Two plant species, *Octonochaeta rosea* and *Octochaetona phillotti*, had a greater plant height in a substrate made of 75% vermicompost (produced by *Perionyx excavates*) and 25% soil than the substrate of a soil amended with chemical fertiliser (Urea and superphosphate) (Reddy & Ohkura, 2004). Singh et al. (2008) found that the dry plant weight of strawberry (*Fragaria* × *ananassa*) was higher when grown in a field sprayed with vermicompost derived from vegetable waste mixed with cow dung at rates of 2.5, 5.0, 7.5, 10 t/ha than a treatment applied with the recommended dose of inorganic fertiliser. Thus, these results indicate that vermicompost has a great potential to be an alternative fertiliser for use in horticultural production.

Furthermore, positive suppression effects of vermicompost on various plant diseases have been found in many plants, including green beans (*Botryitis*), strawberries (*Botryitis*), grapes (*Botryitis*), geraniums (*Botryitis*), tomatoes (leaf spot), *Arabidopsis* (bacterial speck) and apples (powdery mildew) (Arancon, Galvis & Edwards, 2005; Pant et al., 2009). Other effects of vermicompost on reducing water use for irrigation, pest attack and weed growth have been also reported. For example, Arancon et al. (2004) observed significant decreases in the population of aphids and mealy bugs on pepper and mealy bugs on tomatoes, with vermicompost application rates of 20% and 40%. Am-Euras (2009) documented that Cauliflowers applied with vermicompost were almost free of diseases

(95%), while plants grown with chemical fertiliser were susceptible to diseases. Thus, these effects may also indirectly contribute to plant growth and final yield.

Table 2.3 List of parameters of various plants enhanced with vermicompost applications

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*** Adapted from Joshi et al. (2015)**

2.3.2 The nature of vermicompost and changes in plant substrates

Since some remarkable effects of vermicompost on plant growth and development have been found, a number of studies have worked on investigating the factors that lead to these effects. It seems that the effects plant growth attributed to the characteristics of the vermicompost and its interactions with the plant substrate. Many consistent results have shown that the growth-promoting effects of vermicompost are mainly due to the high content of plant available nutrients and the presence of humic acids in it (Joshi et al., 2015).

The high nutritional value of vermicompost has been generally recognised. It has been reported that vermicomposts are rich in nutrients such as nitrates (N), phosphates (P), soluble potassium (K), magnesium (Mg) and calcium (Ca) (Am-Euras, 2009). However, the exact nutrient content of vermicomposts are various, depending on the source of the initial materials. Vermicompost also has advantages over the raw materials and regular compost in terms of nutrient content. Pramanik (2007) found that N content increased by 275%, 178%, 153% and 146% for vermicompost derived from cow dung, municipal solid wastes, grasses and aquatic weeds, respectively, and the content of phosphorus (P) and potassium (K) were also increased, with the greatest increases shown in cow manure (P: 12.70mg/g; K: 11.44mg/g) followed by aquatic weeds, grasses, and municipal solid wastes. Similarly, a study by Agarwal (1999) reported that N, P, K content was increased by three to four times in manure-generated vermicompost (Am-Euras, 2009).

Studies have also shown that the effects of a vermicompost are not purely associated with its nutrient content. It has been found presences of various enzymes such as amylase, lipase, cellulase, and chitinase, and these enzymes have been reported to have effects on breaking up organic matter in the soil. Am-Euras (2009) documented that adequate vermicompost application can significantly improve the activities of soil enzymes including urease, phosphomonoesterase, phosphodiesterase and arylsulphatase (Am-Euras, 2009). Studies have also found the presence of plant growth hormones such as auxins, gibberellins and cytokinins in vermicompost (Edwards et al., 2011). These growth regulators were demonstrated to have been produced by microbes including bacteria, fungi, actinomycetes, yeast and algae. As a result, increased microbial activities in vermicompost (due to the structure of vermicompost and earthworm activities) may attribute to the high content of growth regulators in vermicompost. However, since a high concentration of plant growth regulators may inhibit plant growth, inhibition effects of vermicompost under high application rates may also attribute to this factor (Manh & Wang, 2014). Furthermore, it is noticeable that many studies found that the humic acid extracted from vermicompost can enhance the growth parameters of basil, marigold, pepper, strawberry, tomato and cucumber (Atiyeh et al., 2002; Arancon, Lee, Edwards & Atiyeh, 2003; Befrozfar et al., 2013). Canellas et al. (2010) found both bulk humic acids and different sub-fractions separated from vermicompost induced root growth of maize seedlings, especially for lateral root development. The authors speculated that the growth-promoting effects of humic acid may attribute to the presence of growth regulators which were absorbed by the humates. Beyond these, humic molecules can also interact with the surface of clay to form a complex and stable structure (Pereira et al., 2014) (Figure 2.2). Additionally, the authors pointed out that the fixation can happen both in soil and other sediments, and the changes alter both structure of humic substances and clays/sediments. Thus, the fixation could be one of the factors indirectly contributing to the promoting effects of vermicompost on plant growth.

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Figure 2.2 Interactions between humic substance and clays

* Adapted from Pereira et al. (2014)

The Physico-chemical property of a potting substrate also changed after vermicompost application. Edwards et al. (2011) reported increases in bulk density, container capacity, electrical conductivity, nitrate concentration and overall microbial activity after applying pig manure vermicompost to a potting substrate, MM360, while the air space, total porosity, pH and ammonium concentration in the medium decreased. However, despite some studies having observed these changes, few studies have explained the mechanism of these changes. Further investigation with the interactions between vermicomposts and plant substrates are needed to get maximum benefits from vermicompost applications.

Chapter 3: Feasibility of Combining Municipal and Dairy Industry Waste as Feeding Materials For Earthworm *Eisenia foetida*

3.1 Introduction

The characteristics of raw materials have been considered as the most important factor determining vermicomposting efficiency. Since these raw materials are a food source for earthworms, the characteristics of raw materials can affect earthworm growth and activities. Sim and wu (2010) pointed out that the types of raw material, their constitution and the extent of their decomposition not only influence earthworm growth but also affect the final nutrient state of vermicompost, especially for nitrogen content.

In practice, one common way to modify the characteristics of the raw material used in vermicomposting is to change its constitution by adding amendments or by blending it with other materials (Yadav & Garg, 2010). In the case of this study, the two problematic wastes, green waste-based municipal waste and used animal bedding have both advantages and disadvantages as feeding materials for earthworms. Municipal wastes have advantages on keeping the balance of water and air content in the substrate due to their high fibre content, but they have a relatively low nitrogen content. Used animal bedding, however, is a nitrogen rich waste, because it contains a large amount of dairy cow effluent. Nevertheless, due to its long decomposing time, used animal bedding become very acid, and high mortality rates occurred when earthworms were fed with this waste alone (Table 3.2). Thus, it is likely that combining the two wastes together may have an effect of improving the vermicomposting efficiency of the two types of wastes. However, the effect of mixing two types of wastes will vary depending on the proportions of the two wastes used. Therefore, the objective of the studies in this chapter was to assess how much the earthworm growth and development can be influenced by waste composition and to find the optimum range and proportions of wastes to achieve maximum vermicomposting efficiency.

3.2 Materials and methods

3.2.1 Waste materials

The waste materials used were municipal wastes and used animal bedding.

Municipal waste was obtained from Selwyn District Council and was collected from Pines Resource Recovery Park, 183 Burnham school Rd, Rolleston, Christchurch, New Zealand (S43°35’35.79”, E172°20’22.92”). Three types of waste were used: fresh shredded, hot rotted and old shredded (Figure 3.1). These wastes came from the same source (organic wastes from the local household with a large content of garden waste), but were processed using different methods. Fresh shredded waste (FS) was a fresh green waste shredded in a grinder without additional compost (Figure 3.1: A). (2) Hot Rotted waste (HR) was shredded and composted by thermophilic bacteria in a special container for about a month as showed in Figure 3.1: B. Old shredded waste (OS) (Figure 3.1: C) was just piled up and windrowed outside in the Pine Resource Recovery Park for about 6-7 months.

Figure 3.1 Three types of waste in Pines Resource Recovery Park

* A: Fresh shredded waste (FS); B: Hot rotted waste (HR); C: Old shredded waste (OS)

Used animal bedding (UAB) waste also used in this study, which was collected from a dairy farm in Eyrewell, Canterbury, New Zealand (S43°25’44.84”, E172°19’39.56”). The bedding, originally of small pieces of wood chips, have been mixed with dairy cow slurry during its uses (Figure 3.2). The waste had been piled outside of farm building in Eyrewell and left for several years before being used in the experiment.

Figure 3.2 Used animal bedding (UAB) from Eyrewell

3.2.2 Source of earthworms

Earthworms were obtained from a commercial earthworm farm, WormsRUs (53A Clark Rd, Karaka, Auckland 2580), and were maintained under laboratory conditions for a month for acclimation before the experiments begin.

The species used was an epigean earthworm, *Eisenia foetida*. This is one of the most commonly used earthworm species for vermicomposting owning to its tolerance of a wide range of environmental conditions and its high metabolic rate (Yadav & Garg, 2011). The life cycle characteristics and culture requirement for the species are presented in table 3.1.

Table 3.1 Life cycle characteristics and culture requirement of *Eisenia foetida*

	Eisenia foetida
Mode of reproduction	Pathenogenetic
Growth to maturity (days)	28-30
Cocoon incubation (days)	18-26
Cocoon viability (%)	73-80
Number of cocoon per day	0.35-0.5
Optimal moisture content (%)	80-85
Optimal temperature (°C)	25

Adapted from Lowe et al. (2014).

3.2.3 Physical and chemical analyses

3.2.3.1 Moisture content

The moisture content of waste materials and vermicompost were calculated using the formula as described by Suthar (2007):

$$\text{Moisture content} = \frac{\text{wet weight - dry weight (water content in the waste)}}{\text{wet weight}} \square 100\%$$

The dry weight of vermicompost and waste was determined by placing a sample in an oven for 24 h at 105°C.

3.2.3.2 Analysis of pH and electrical conductivity

The pH of wastes and vermicomposts were measured in suspension with water, using 5g of ground samples and 25ml of deionized (DI) water. Samples were shaken for 5 mins and centrifuged at 1500 rpm for 15 minutes before analysis and were measured using a pH meter (S20 SevenEasy™ pH; Mettler- Toledo, Switzerland) (Blakemore et al., 1987). After pH analysis, the same samples were used to measure electrical conductivity using a Radiometer (Copenhagen CDM 83, Denmark).

By measuring electricity conductivity of samples, total soluble salts could also be calculated using the equation (Blakemore et al., 1987):

K_{25} (millimho/cm) × 0.35 = Total soluble salts (%)

3.2.3.3 Analysis of total carbon and nitrogen

The analysis of total carbon and Nitrogen content were operated by a professional technician. Four gramme subsamples were weighted and analysed using a CNS Elemental Analyser following Dumas combustion methods.

3.2.3.4 Analysis of ammonium-N ($NH4^{+}$-N) and nitrate-N ($NO3^{-}$-N)

The sieved (2mm) samples of vermicompost from Vermicomposting Experiment 1 were air dried and transported to the laboratory for analysis. A subsample of vermicompost was dried at 105°C for measuring the moisture content of each Vermicompost. Four grammes subsamples were weighted and shaken with 40 mL of 2 M potassium chloride (KCL) for 1 hour. Thereafter, the samples were centrifuged at 2000 rpm for 10 minutes and filtered using filter paper (Whatman No. 41) (Blakemore et al. 1987). The extracts were then frozen before analysed by a Flow Injection Analyser (FIA) (FOSS FIA star 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden) for ammonium-N ($NH4^{+}$-N) and nitrate-N ($NO3^{-}$-N).

3.2.4 Statistical analysis

Data from the experiment were processed and analysed using JMP (SAS) and Excel speadsheet programme (Microsoft 2011). Effects from types of municipal waste, the percentage of UAB and their interactions with earthworm growth, reproductive rate and survival rate were assessed using two-way analysis of variance (ANOVA), respectively. Additional post-hoc tests, Tukey’s HSD test (Vermicomposting Experiment 1) and Student’s t-test (Vermicomposting Experiment 2) were also used to check the significance among all treatments.

3.2.5 Vermicomposting Experiment 1

The objective of the experiment was to examine the feasibility of mixing three types of municipal waste and used animal bedding as feeding substrates for earthworms in vermicomposting, and to investigate if there are positive effects of mixing waste on earthworm growth parameters (eg. survival rate, biomass and reproductive rate). The experiment also assessed the changes of these growth parameters in an enclosed vermicomposter during the experimental period and explored the optimal range of wastes for earthworm growth.

3.2.5.1 Treatments and experimental design

The experiment was conducted in the Ecology Laboratory in Burns Building, Lincoln University, Christchurch, New Zealand. The experiment lasted for 90 days from 26 August 2015 to 24 November 2015, and the temperature during the experimental period ranged from 18.1°C to 21.3°C.

Plastic containers (15cm×15cm×8.9cm; 2L) with perforated lids were used for raising earthworms. Each container was filled with 100g feeding materials (based on dry matter), and the moisture content of the feeding materials was adjusted to 70%. Thirteen adult earthworms with weight at 10±0.2g were placed into each container. The moisture content in the container was maintained at 70±5% by periodic sprinkling with water throughout the study period. Three ratios of dairy industry waste (used animal bedding, UAB) at 5%, 10%, 20% were added to three types of municipal wastes (FS, HR, OS) as feeding mixtures to test the compatibility of the two waste types: 1) 5% of UAB + FS, HR, OS respectively; 2) 10% of UAB + FS, HR, OS respectively; 3) 20% of UAB + FS, HR, OS respectively; Control: 100% of FS, HR, OS. Each treatment was replicated six times.

3.2.5.2 Experimental procedure and assessment

The vermicomposting process was undertaken in the container during the experimental period. No extra feeding materials were added during this time. The growth rate, survival rate and cocoon production rates were calculated by regularly weighing earthworms and counting the number of earthworms and cocoons in each container.

Owning to the limitation of the earthworm life cycle, the experiment was assessed four times in total (Figure 3.3). The first and second checking were at the 10^{th} day and the 20^{th} day after the experiment set up, respectively. On the first two occasions, earthworm biomass, numbers of earthworms and cocoons were all measured. On the 30^{th} day, since earthworm cocoons had started hatching, it was impossible to check the total number of cocoons in the system, and newly born earthworms were too fragile to tolerate the disturbances from turning over the substrate. Thus, only the weight and number of adult earthworms were measured from then on. At the end of experiment, weight of earthworms, a total number of parent earthworms and newly hatched earthworms were recorded. The Vermicompost in the containers were then harvested and sieved (2mm) to remove earthworms and undigested waste. The harversted homogeneous vermicompost was then stored separately by type for further experimentation.

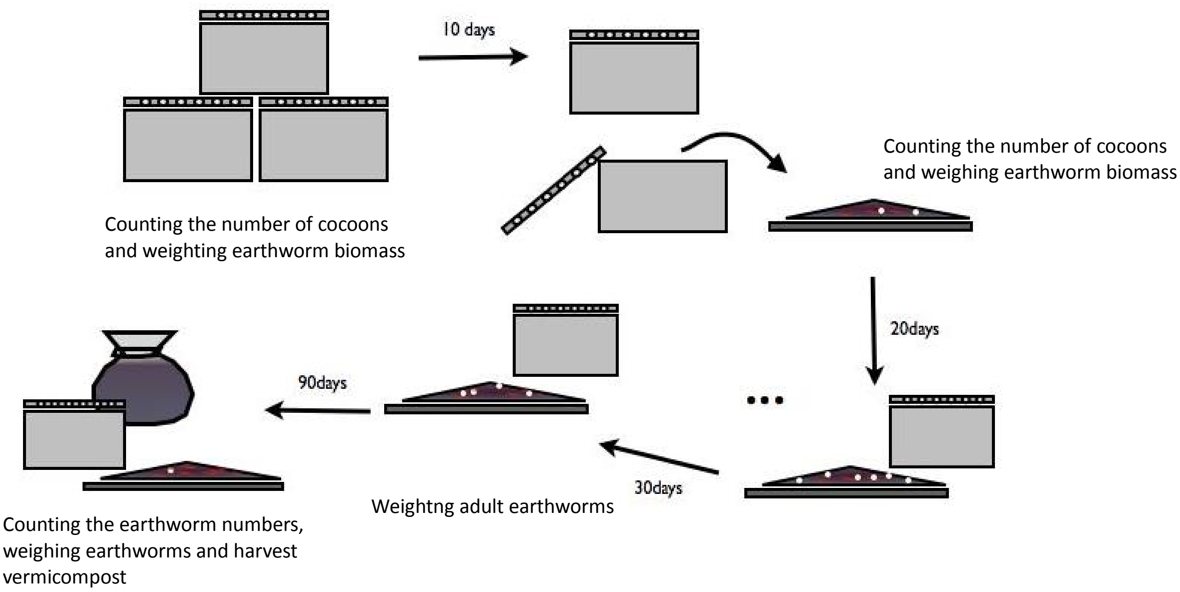

Figure 3.3 Diagram showing procedure and assessments of Vermicomposting Experiment 1

3.2.6 Vermicomposting Experiment 2

3.2.6.1 Treatments and experimental design

The second experiment was conducted in the ecology lab at Lincoln, the same as the first experiment.

This experiment consists of two parts. The first part was a preparatory experiment for producing cocoons used in the experiment that followed, and it started from 9 September 2015. The second part was the main experiment for assessing the influence of different waste compositions on cocoon hatching and growth of young earthworms. It ran from 14 September 2015 until 17 November 2015.

In the pre-experiment of Vermicompost Experiment 2, the same plastic containers (2L) with perforated lids as in the Vermicomposting Experiment 1 were used to produce cocoons. Fresh shredded waste with adjusted moisture content at 70% was added into the container as raising substrate. A petri dish (90mm×25mm; diameter×depth) with 40g of waste in different proportions was used in the following part of the experiment. The moisture content of waste materials in the Petri dish was adjusted and maintained at 70%. As negative effects of old shredded (OS) on earthworm growth have found in the Vermicompost Experiment 1, this experiment was designed to only test the effects of fresh shredded waste (FS) and hot rotted waste (HR) mixed with different proportions of used animal bedding (UAB) on the development of cocoons. There were five ratios of UAB were added to FS and HR respectively: 1) 10% UAB + FS; 2) 10% UAB +HR; 3) 20% UAB +FS; 4)

20% UAB +HR; 5) 30% UAB + FS; 6) 30% UAB+ HR; 7) 40% UAB + FS; 8) 40% UAB +HR; 9) 50% UAB + FS; 10) 50% UAB +HR. Each treatment was replicated three times.

3.2.6.2 Experimental procedure and assessment

In the preparatory experiment, five plastic containers containing 50g earthworms and 200g bedding substrate (FS) were left for 4 days without disturbance. On the fifth day, the substrate with earthworms was turned over, and cocoons from the five containers were removed gently by hand using a pair of forceps. The collected cocoons were immediately put into the Petri dishes. Four average-sized cocoons were randomly placed in the middle of each dish and lightly covered with substrate. These Petri dishes were then put in a lightproof box to keep Petri dishes in dark condition. Records of cocoon development were taken regularly during the experimental period. After 65 days, the numbers of hatched cocoons, the numbers of hatched earthworms and the growth status of earthworms were recorded at the end of the experiment.

3.3 Results and discussion

3.3.1 Materials

3.3.1.1 Raw materials

Basic physico-chemical characteristics of the three types of municipal wastes (fresh shredded waste; FS, Hot rotted waste; HR, Old shredded wastes, OS) and used animal bedding (UAB) are shown in table 3.2.

For all wastes, with an increase of decomposition time (decomposition time: FS < HR < OS), there was a decrease in moisture content (MC), pH, total carbon (TC), and carbon/nitrogen ratio (C: N), but an increase in electricity conductivity (EC). However, in the case of total nitrogen content (TN), OS had the highest nitrogen percentage (2.19%) followed by FS (2.10%) and HR (1.80%). These differences in MC, TC, TN and EC may have been resulted from microbial activities, as microbes need water and a source of carbon and nitrogen to mineralize organic matter during the composting process. In the normal composing process, pH falls below neutral at the beginning due to the formation of organic acid and rises above neutral due to the consumption of acids and the production of ammonium (Beck-Friis et al., 2003). Thus, the acid pH found here may reflex the higher content of organic acids, and also indicated that the decomposing process of all types of municipal waste was still in the initial phase.

Physico-chemical characteristics of UAB (used animal bedding), however, were very different from the municipal wastes. It had significantly higher MC, TC and TN, with a lower pH. The EC and salt content of UAB were also quite high at about 3.05 mS/cm (1.07%) similar to that of the OS (EC: 3.31 mS/cm; Total soluble salts: 1.16%). The high TC of UAB may be due to the character of the waste which is mainly made up of wood chips. The high MC, EC and TN indicate that the UAB contains quite a high level of cow effluent. The low pH of UAB probably resulted from the poor aeration of the waste at the initial stages, as it was piled quite high outside leading to the accumulation of organic acids, which could be a food source for some microorganisms. Generally, however, these acids inhibit the overall activities of microbes. Organic acids were demonstrated to be the main influencing factor of the composting process by affecting the transition from a mesophilic level to a thermophilic level (Sundberg & Jonsson, 2003). This probably explains the low decomposition rate of the UAB from Eyrewell.

Table 3.2 Physico-chemical characteristics of initial wastes (mean±SEm, n=3)

Waste Types	MC (%)	EC (mS/cm)	pH	TC (%)	TN (%)	C:N
FS	31.41±4.73	1.63±0.006	7.3 ±0.019	38.03±0.60	2.10±0.02	18.15±0.11
HR	13.41±0.48	2.19±0.009	6.97±0.003	29.21±0.31	1.80±0.01	16.25±0.09
OS	10.06±0.17	3.31±0.009	6.24±0.003	26.67±0.32	2.19±0.02	12.16±0.05
UAB	56.46 ±2.02	3.05±0.019	4.37±0.003	80.53±0.79	3.64±0.04	22.16±0.24

* Values of TC and TN is on dry weight basis

3.3.1.2 Vermicomposts

Table 3.3 shows the physico-chemical characteristics of vermicompost derived from different sources. Generally, the pH of vermicompost decreased with the increased amount of UAB in the initial feeding material for all types of waste, and the pH value of vermicomposts were all higher than the initial materials. The values of EC for all vermicomposts was about 2 mS/cm, which tended to be neutral in comparison with the initial waste materials. For OS treatments, there was a clear trend that the EC of final vermicompost was reduced with increasing proportions of UAB, and the EC of the OS-based vermicomposts (2.02-2.85 mS/cm) were lower than the initial wastes.

In the case of the nutrient quality of vermicompost, the HR-based vermicompost generally had the lowest N content, which may have been due to the lowest N content in HR compared to FS and OS. Vermicomposts derived from 0%UAB+FS and 20%UAB+FS had the highest N content at 80.70 mmol and 73.94 mmol (NH_4-N + NO_3-N), respectively. In terms of the forms of N in vermicompost, it was noticeable that 20%UAB addition altered the quality of vermicompost differently for the three municipal wastes. For fresh shredded waste (FS) with a substitution of 20% UAB had a greater ammonium-N content (57.04 mg/l) compared to the treatment with 10% UAB rate (6.89 mg/L), but lower nitrate-N content. The hot rotted waste (HR), however, showed a slight decrease in ammonium-N content but an increase in nitrate-N with the increased rates of UAB substitution. The old shredded waste (OS) produced a vermicompost which was both significantly lower in the content of ammonium-N and Nitrate-N especially when OS substituted with 20% UAB.

In regard to the N cycle (Figure 3.4) in the vermicomposting system, we could find that in the FS-based vermicomposting system, additional 20%UAB substitution actually inhibited nitrification process, and slightly increase the ammonification process. However, in the HR-based system, the nitrification process seems to be promoted under 20% UAB substitution. For the OS-based vermicomposting system, the lower N content indicated that there is a great loss of nitrogen in the system. It is possible that the losses resulted from N denitrification, as the N consumption in the OS system was less than in the FS and HT system due to the lower growth and reproductive rate of earthworms (3.2.2). Thus, it is hard to clarify the function of UAB addition on the N status of vermicompost due to the complex interactions among microorganisms. Nevertheless, it is clear that the N status in the final vermicompost has a correlation with the raw materials used. There may be positive effects on the final nutrient status of vermicompost with additional UAB substitution, but negative effects (reduced total N content) could also occur. In addition, it is still not clear how the characteristics of UAB altered the N cycle in the vermicomposting system and how these characteristics interact with these municipal wastes. Future works are required to study on this aspect.

Table 3.3 Physico-chemical characteristics of vermicompost produced from Vermicomposting Experiment 1 (mean±SEm, n=3)

Treatment	pH	EC (mS/cm)	Ammonium-N (μg/g)	Nitrate-N (μg/g)
0%UAB+FS	8.19 ± 0.124	2.25 ± 0.006	6.26 ± 0.458	335.86 ± 14.462
5%UAB+FS	8.19 ± 0.012	2.14 ± 0.007	6.95 ± 0.291	226.96 ± 24.766
10%UAB+FS	8.00 ± 0.009	2.27 ± 0.007	6.89 ± 0.556	201.00 ± 14.839
20%UAB+FS	7.75 ± 0.010	2.35 ± 0.009	57.04 ± 2.580	131.06 ± 19.074
0%UAB+HR	8.20 ± 0.009	2.48 ± 0.009	12.73 ± 0.472	25.17 ± 0.707
5%UAB+HR	7.90 ± 0.007	2.82 ± 0.006	16.00 ± 0.265	39.35 ± 1.922
10%UAB+HR	7.91 ± 0.038	2.05 ± 0.003	7.51 ± 0.412	75.49 ± 2.006
20%UAB+HR	7.71 ± 0.033	2.25 ± 0.003	6.40 ± 0.202	107.02 ± 8.292
0%UAB+OS	8.20 ± 0.014	2.85 ± 0.003	5.52 ± 0.213	259.62 ± 4.004
5%UAB+OS	7.95 ± 0.023	2.58 ± 0.003	5.50 ± 0.102	207.24 ± 1.911
10%UAB+OS	8.04 ± 0.006	2.41 ± 0.003	8.73 ± 0.387	204.61 ± 13.513
20%UAB+OS	7.65 ± 0.010	2.02 ± 0.008	4.00 ± 0.583	88.43 ± 0.638

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Figure 3.4 Microbiological nitrogen cycle

*Adapted from Tihomson et al. (2012).

3.3.2 Earthworms

3.3.2.2 Growth and reproduction

Growth and fecundity parameters recorded in the first and second ten day are presented in Table 3.4 and Table 3.5. It is noticeable that the cocoon production rate in OS-based vermicomposting system (old shredded waste) was significantly lower than FS-based (fresh shredded waste) and HR-based (hot rotted waste) system in the first ten days (0.09-0.25 compared to 0.36-0.46). In addition, the negative effects of OS on earthworm survival and growth had shown in the first ten days although the differences are mostly not significant.

Table 3.4 Growth and fecundity parameters in the first ten days (mean±SEm, n=6)

Treatment	Number of survived earthworms	Average weight of earthworm (g)	Cocoon production rate (per earthworm per day)
FS	13.00 ± 0.000 a	0.80 ± 0.019 ab	0.40 ± 0.027 a
FS+5%UAB	12.22 ± 1.633 ab	0.83 ± 0.037 a	0.44 ± 0.055 a
FS+10%UAB	13.00 ± 0.000 a	0.86 ± 0.034 a	0.36 ± 0.037 a
FS+20%UAB	13.00 ± 0.000 a	0.86 ± 0.016 a	0.36 ± 0.031 a
HR	12.80 ± 0.447 a	0.82 ± 0.018 a	0.42 ± 0.053 a
HR+5%UAB	12.50 ± 0.837 ab	0.84 ± 0.030 a	0.46 ± 0.040 a
HR+10%UAB	13.00 ± 0.000 a	0.84 ± 0.030 a	0.37 ± 0.033 a
HR+20%UAB	12.50 ± 0.837 ab	0.82 ± 0.020 a	0.37 ± 0.039 a
OS	11.33 ± 2.875 b	0.73 ± 0.053 b	0.11 ± 0.053 b
OS+5%UAB	12.67 ± 0.817 a	0.80 ± 0.021 ab	0.10 ± 0.026 b
OS+10%UAB	12.17 ± 1.169 ab	0.80 ± 0.027 ab	0.09 ± 0.019 b
OS+20%UAB	13.00 ± 0.000 a	0.82 ± 0.019 a	0.15 ± 0.046 b

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t-test), and indicated by different letters.

In the second ten days, the cocoon production rates are higher in all treatments than the first ten days. Nevertheless, the disadvantage of OS on earthworm reproduction had been more obvious. In case of earthworm growth, the average weight of earthworms all declined at the day 20 compared to the day 10 and even below the initial weight (0.77g/ earthworm). On the other hand, the content of UAB also affected the earthworm growth and reproduction especially for FS and HR. For instance, 20% UAB substitution of FS and HR results in significant improvements on cocoon production rate in the second ten days compared with the treatments without UAB substitution (0.58 to 0.46, 0.52 to 0.30, respectively).

Table 3.5 Earthworm growth and fecundity parameters recorded at day 20 (mean±SEm, n=6)

Treatment	Number of survived earthworms	Average weight of earthworm (g)	Cocoon production rate (per earthworm per day)
FS	12.67 ± 0.333 ab	0.65 ± 0.019 b	0.46 ± 0.028 b
FS+5%UAB	12.33 ± 0.667 ab	0.65 ± 0.031 b	0.52 ± 0.032 ab
FS+10%UAB	12.67 ± 0.211 ab	0.64 ± 0.028 b	0.49 ± 0.031 ab
FS+20%UAB	13.00 ± 0.000 a	0.65 ± 0.018 b	0.58 ± 0.032 a
HR	12.50 ± 0.342 ab	0.72 ± 0.038 a	0.30 ± 0.048 c
HR+5%UAB	12.50 ± 0.342 ab	0.69 ± 0.015 ab	0.46 ± 0.051 b
HR+10%UAB	12.83 ± 0.167 a	0.67 ± 0.017 ab	0.47 ± 0.041 ab
HR+20%UAB	12.17 ± 0.401 ab	0.68 ± 0.011 ab	0.52 ± 0.051 ab
OS	11.17 ± 1.33 b	0.70 ± 0.034 ab	0.14 ± 0.035 d
OS+5%UAB	11.83 ± 0.543 ab	0.74 ± 0.014 a	0.15 ± 0.038 d
OS+10%UAB	11.83 ± 0.654 ab	0.69 ± 0.032 ab	0.17 ± 0.031 d
OS+20%UAB	13.00 ± 0.000 a	0.69 ± 0.012 ab	0.14 ± 0.019 d

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t-test), and indicated by different letters.

* The cocoon production rate was calculated by the mean of cocoon produced in a period of ten days from 10th to 20th day.

Figure 3.5 shows the changes in average earthworm biomass during the experimental period on different municipal waste-based vermicomposting systems, regardless of the content of UAB. Overall, all earthworms lost over half of their initial weight by the end of the experiment, despite there being a slight increase in earthworm biomass during the first ten days for the FS and HR. Earthworms on FS-based vermicomposting system seem to lose their weight faster, followed by the HR-based and OS-based worms. In contrast, the reproductive rates of the OS worms were significantly lower than the FS and HR worms (Table 3.4 and Table 3.5; Figure 3.9). Thus, the greater weight loss of FS and HR worms may be due to the higher reproductive rate as more energy may be needed to support the multiplication.

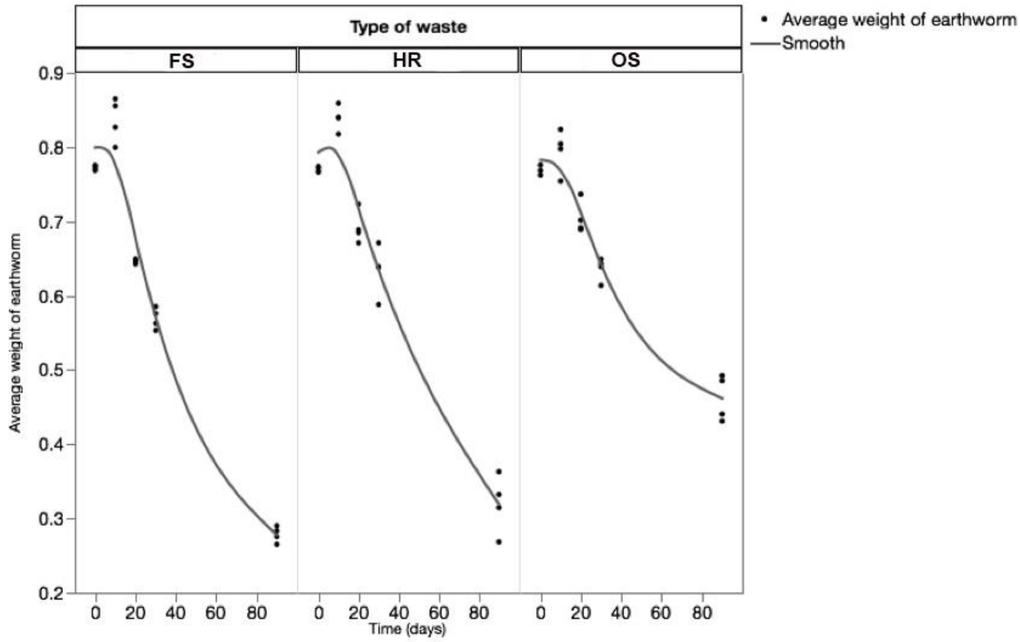

Figure 3.5 Influences of type of municipal waste on earthworm biomass during the experimental period

* The type of waste refers to the three different types of the organic municipal wastes studied in the research.

* FS: fresh shredded waste; HR: hot rotted waste; OS: old shredded waste.

3.3.2.1 Mortality rate

In addition to initial materials affecting earthworm growth and reproduction, They can also influence earthworm survival rate. It appeared that fresh shredded waste was more suitable as feeding substrate for earthworms, and mortality rates were lower in FS compared to HR and OS (Figure 3.6). It was also found that the mortality rates were higher in the early period of the experiment and tend to be stable after 30 days. This indicated that the worms gradually adapted to the new feeding material after day 30. Thus, the high mortality rates in the early part of the experiment may have been due to the acclimation of earthworms.

On the other hand, proportions of UAB in the initial material had effects on earthworm survival rates. The survival rates of earthworms increased with the content of UAB (up to 20%) in feeding materials, regardless of the type of municipal waste (Figure 3.7). However, when looking at both effects of the waste type and UAB content, the effects of the UAB addition on earthworm survival only showed when it combining with FS and OS (Figure 3.8). It is not known what is the factor leading to these effects is, but it was probably due to the high air content, greater N source or the absorption ability of UAB.

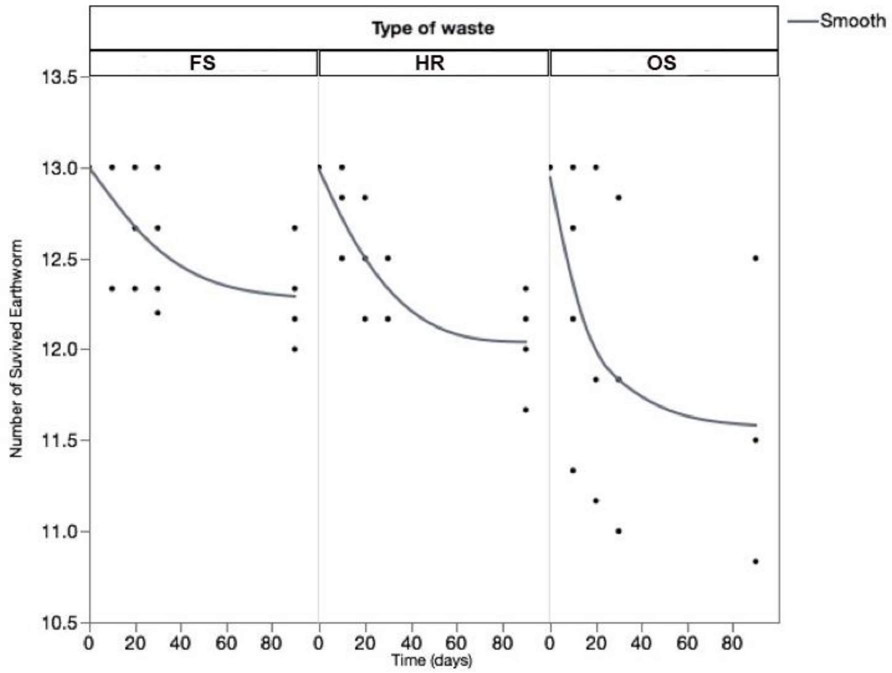

Figure 3.6 Influences of type of municipal waste on earthworm survival

* FS: fresh shredded waste; HR: hot rotted waste; OS: old shredded waste.

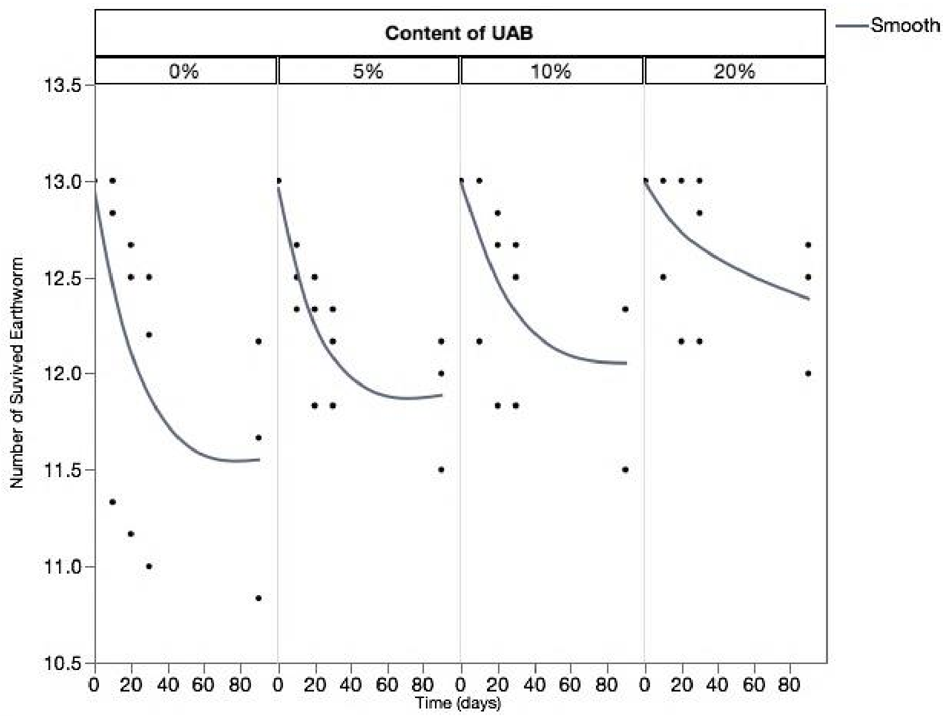

Figure 3.7 Effect of UAB content on earthworm survival

* Content of UAB refers to the percentage of used animal bedding content in a waste mixture.

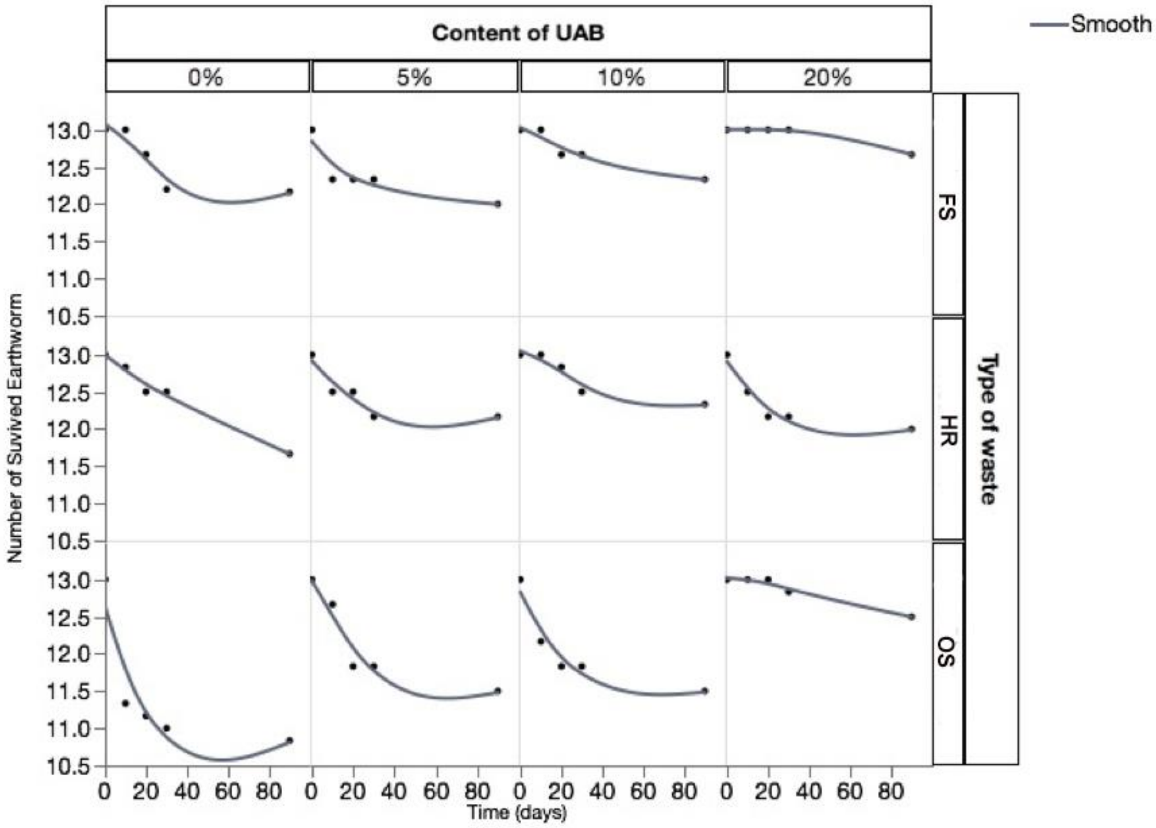

Figure 3.8 Changes in numbers of earthworms surviving for all treatments

* FS: fresh shredded waste; HR: hot rotted waste; OS: old shredded waste.

3.3.2.4 Cocoon hatching

At the end of the experiment (90 days), the number of newly generated earthworms was counted and is shown in Figure 3.9. Generally, FS produced more juvenile earthworms than HT and OS. The treatment with the highest number of junior earthworms was FS + 5%UAB with 334 new earthworms on average, and it was almost 25.7 times more than the initial number of earthworms (13). Fresh shredded waste alone without UAB addition also produces quite a high number of junior earthworms, at about 327, followed with FS+10%UAB and FS+20%UAB at about 295 and 267 worms respectively. The highest earthworm production in the HR-based treatments were HR+5%UAB at 213 worms followed by HR+20%UAB, HR+10%UAB, and HR (187, 174 and 135 worms, respectively). OS-based treatments, however, produced a significantly lower number of junior earthworms with a range of 60-71.5 worms.

Despite that the number of newly generated earthworms was quite high especially for FS treatments, the growth and development of these new earthworms were delayed for all treatments, due to the lack of available nutrients for new earthworm growth in these vermicomposting systems. Also, the number of junior earthworms seems consistent with cocoon production rates in the systems (table 3.4, table 3.5). However, the effects of feeding material on earthworm cocoon hatching rates and the number of earthworms hatched from each cocoon could not be quantified in this experiment. Therefore, another experiment was set up to investigate the influence of feeding material on second generation earthworm growth and development.

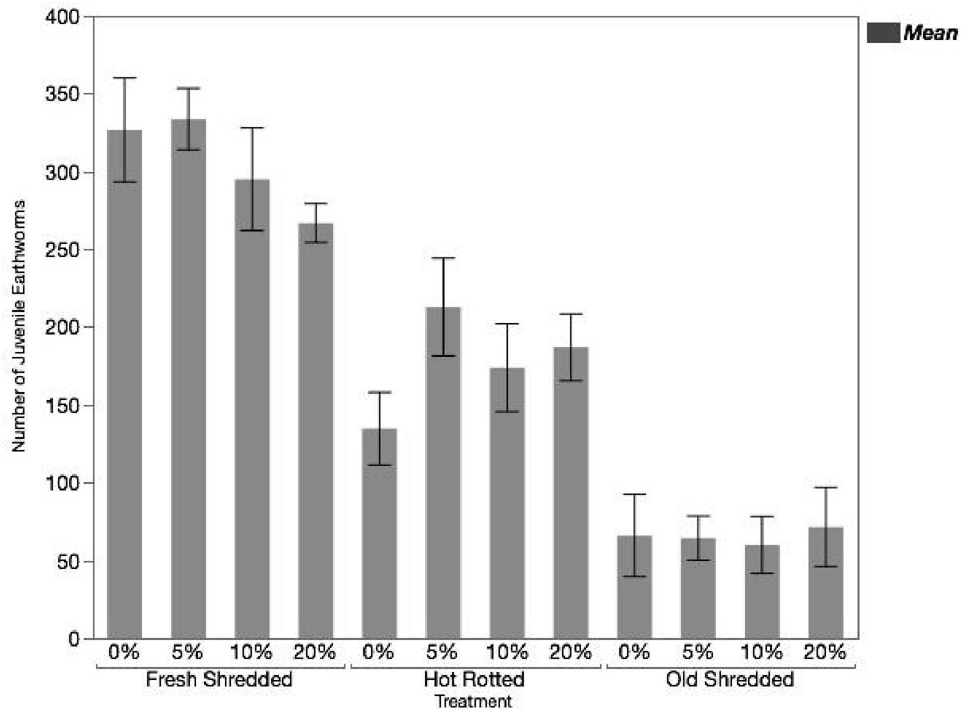

Figure 3.9 Mean number of newly generated earthworms recorded at the end of the experiment

* The percentages (0%, 5%, 10% 20%) are equal to the proportion of UAB in initial earthworm feeding materials and the error bar is based on standard error of mean (SEm).

* Each error bar is constructed using standard error from the mean.

3.3.3 The second earthworm generation

Since the composition of feeding material may also affect cocoon hatching and development, an additional experiment, Vermicomposting Experiment 2, was set up to test the effects of feeding material on the growth and development of cocoons and newly generated earthworms.

The cocoon viability, the number of newly generated earthworms and the average number of earthworms per cocoon is shown in Figure 3.10, Figure 3.11, and Figure 3.12.

For cocoon viability, it can be seen from Figure 3.10 that FS-based (fresh shredded waste) treatments generally have a higher cocoon hatching rate than HR-based (hot rotted waste) treatments. In addition, the cocoon hatching rate in FS-based treatments was generally in the range of optimal cocoon viability (73-80%) or even greater (Table 3.1)(Lowe et al, 2014), apart from the treatment of FS+10%UAB and FS+50%UAB treatment with cocoon hatching rate of 58.3% (2.33) and 50.0% (2), respectively. In contrast, the hatching rate with the HR-based treatments was nearly all below the optimal range, apart from the HR+40%UAB, which was at 75.0% (3). It was noticeable that there was a severe drop (about 50%) in cocoon hatching rates when the proportion of UAB increased from 40% to 50% for both types of municipal waste (FS: 3.67 to 2, HR: 3 to 1.33).

A similar trend was also seen in the total number of newly generated earthworms (Figure 3.11). Nevertheless, some differences between the two graphs were still quite obvious. First, the gaps between each treatment tended to increase. For example, when the proportion of UAB increased from 40% to 50%, the total number of newly generated earthworms decreased from 7.67 to 3 (61%) and 5.33 to 1.33 (75%) and for FS and HR respectively, while the decrease in cocoon viability was just about 50%. Additionally, for HR-based treatments, there was a clear tendency for the total number of hatched earthworms to increase with UAB addition up to 40%. However, the negative effects started to show on cocoon hatching when UAB was increased to 50% of the initial earthworm feeding substrate.

Fig 3.12 illustrates how initial feeding substrates affect the number of earthworm hatching from each cocoon. As it can be seen from the graph, the number of earthworms hatching from each cocoon was mostly within a range of 1.5-2.5 earthworms per cocoon, except for FS+50%UAB, HR, and HR+50%UAB treatments. These three treatments were also the treatments which had the most negative effects on cocoon viability and the number of total newly generated earthworms. Thus, based on these results of the experiment, it could be speculated that there may be some toxic compounds or unfavourable characteristics for earthworm cocoon development in the municipal waste, especially for the HR. Also, it seems that UAB addition may partly diminish the negative effects of HR on cocoon development, while the effects of UAB addition to FS, however, were more variable. Nevertheless, an excess amount of UAB addition may also have an adverse impact on cocoon development. Overall, despite that the negative effects of HR on cocoon hatching diminished by mixing with UAB, apparently, the FS is still the most suitable type of municipal waste to use in vermicomposting with regard to its effects on earthworm cocoon development.

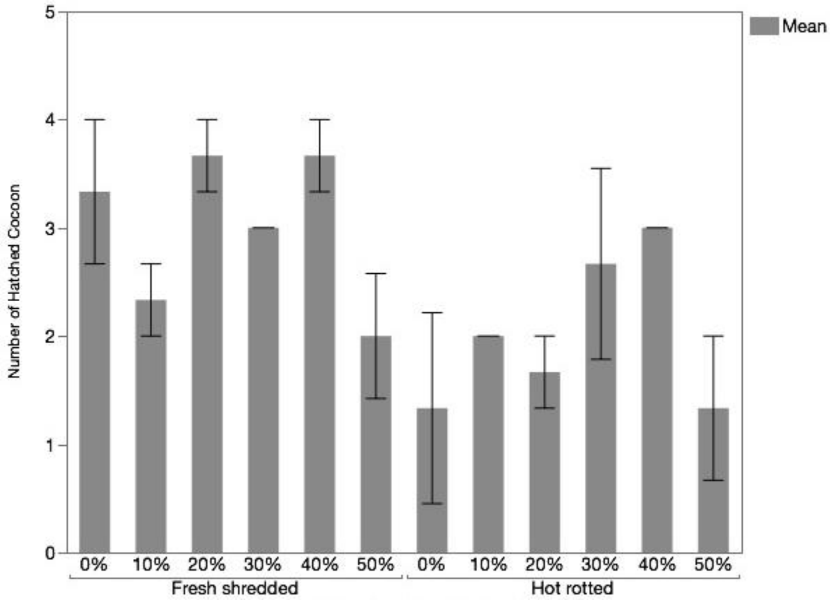

Figure 3.10 Cocoon viability in all treatments

* The percentages (0%-50%) are equal to the proportion of UAB in initial earthworm feeding materials and the error bar is based on standard error of mean (SEm).

* Each error bar is constructed using standard error from the mean.

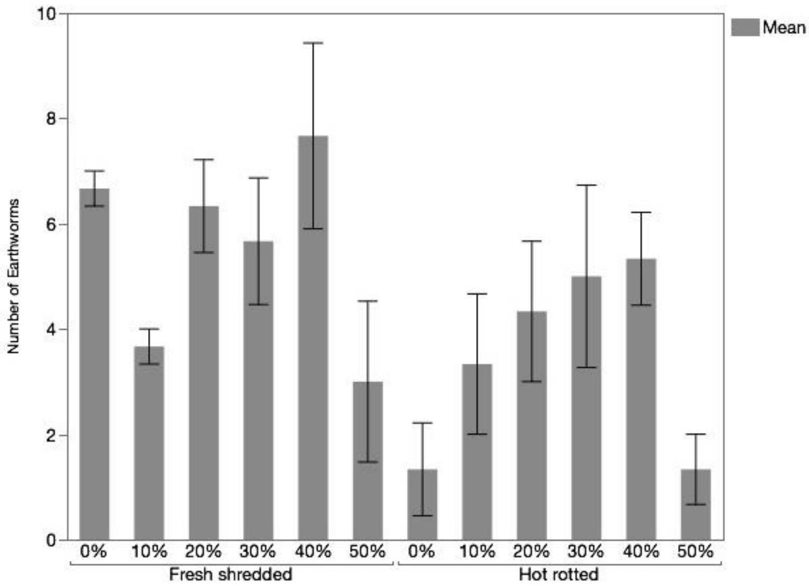

Figure 3.11 Total number of hatched earthworms in all treatments

* The percentages (0%-50%) are equal to the proportion of UAB in initial earthworm feeding materials and the error bar is based on standard error of mean (SEm).

* Each error bar is constructed using standard error from the mean.

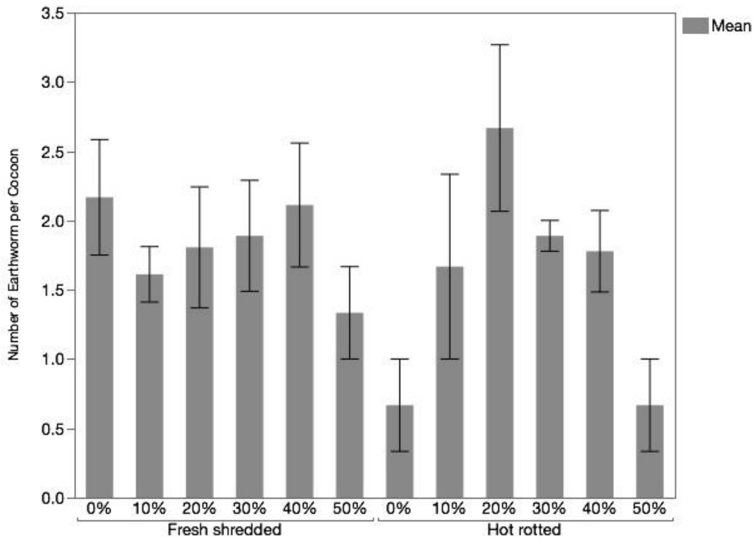

Figure 3.12 Mean number of earthworms hatched from each cocoon in all treatments

* The percentages (0%-50%) are equal to the proportion of UAB in initial earthworm feeding materials and the error bar is based on standard error of mean (SEm).

. * Each error bar is constructed using standard error from the mean.

In order to have an overview of earthworm growth for all treatments, the newly generated earthworms were classified into one of five stages according to their body size and development (for details see the annotation of the Figure 3.13). The number of earthworms belonging to each stage was recorded, and most of the earthworms, as shown in Figure 3.13, were in the scope of stage 3. However, these earthworms actually had grown on the substrate for about 63 days from cocoons, which exceeded the time that *Eisenia foetida* needs to complete its life cycle (46-56 days) under optimal conditions (Lowe et al, 2014). Thus, this actually indicated that the growth and development of earthworms in these treatments were all delayed by unknown stress. In addition, when looking at the number of larger earthworms (stage 4 and stage 5), it is interesting to note that all treatments with UAB addition had earthworms reaching stage 4, while the treatments of pure FS and HR did not find any earthworms reaching stage 4. Thus, it could be speculated that the promotion effects on earthworm growth with UAB addition may be partly linked to the greater nitrogen content of UAB as earthworms need nitrogen to grow their body. For treatments with 50% UAB substitution, despite adverse effects on cocoon development having been found in the treatments, there did not seem to be obvious inhibiting effects on earthworm growth, since large earthworms (stage 4) were found in both FS+50%UAB and HR+50%UAB.

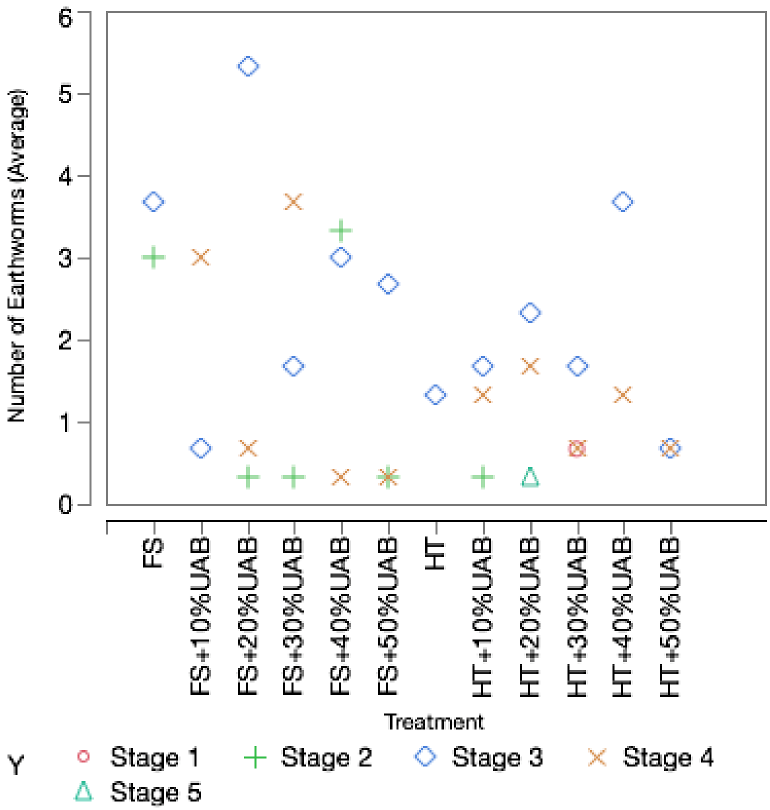

Figure 3.13 Growth status of newly generated earthworms in all treatments

*The different stages of earthworm growth are defined as below: stage 1 (○): Referring to Earthworms in a stage that looks like newborn baby worm with transparent or light pink colour and the body size less than 1cm ; Stage 2 (+): Referring to earthworms in a stage with the body length ranged from 1 to 2 cm; Stage 3 (⋄): Referring to earthworms in a stage that have grown in a long body, but still very thin; Stage 4 ( ): Referring to earthworms in a stage that have had a similar body size of adult earthworm but without visible clitellum; Stage 5 (△) Referring to adult earthworms with visible clitellum.

Chapter 4: Plant Growth Responses of Vermicompost

4.1 Introduction

Agricultural application of organic matter to soil has long been recognized as an effective method for maintaining soil fertility and supporting crop growth. Organic matter addition has been reported to accelerate soil microbial activities with increased values of biomass C, basal respiration, biomass C to total organic C ratios, and metabolic quotients (Edwards et al., 2011). Moreover, organic matter consistently releases plant-avaiable nutrients with increased microbial activities. Thus, the properties of agricultural soil can be changed physically, chemically and biologically in favour of plant growth.

Many crop yields have been found to have a positive response to organic matter addition following the law of diminishing returns (Tittarelli et al., 2007; Hose et al., 2014). Vermicompost as a form of organic fertiliser can function equally as organic matter, and can even perform better than regular compost owning to its unique characteristics. Vermicompost is physically described as an homogenous, peat-like material with higher porosity, aeration, drainage and better water-holding capacity than regular compost. It is also a more nutrient-intensive organic fertiliser than conventional compost, and most of the nutrients such as nitrates (N), phosphates (P), exchangeable phosphorus (P), soluble potassium (K), magnesium (Mg) and calcium (Ca) (Am-Euras, 2009) are in a plant available form. Additionally, vermicompost had been found to contain much larger populations of bacteria (5.7×10^{7}), fungi (22.7×10^{4}) and actinomycetes (17.7×10^{6}) than conventional thermophilic composts (Nair et al., 1997). Because vermicompost has such outstanding physical, chemical and biological properties, it has the potential to be an excellent material for use as a soil or substrate amendments for plant growth.

Much research has shown positive effects of vermicomcompost as an amendment on various plant growth parameters (Joshi et al., 2015). However, the results from these studies are not consistent due to differences in plant species used, source and volume of vermicompost, cultural conditions and application methods. However, it is likely that these factors may indirectly affect the effectiveness of vermicompost as a plant fertiliser under different conditions as each factor may have complex interactions with others. Therefpre, there is a need to test plant responses to particular types of vermicompost in order to investigate the optimal method of utilisation.

The aims of the research reported in this chapter were to evaluate how plants respond to addition of waste-derived vermicomposts, and to investigate the factors affecting plants responses.

4.2 Materials and methods

4.2.1 Experimental site

All the experiments in the chapter were conducted in the Aluminex glasshouse at Lincoln University, Canterbury (S43°38′43.29″, E172°27′43.81″) (Figure 4.1). The overall pot trail consisted of six experiments carried out for a period of 72 days. The glasshouse had an inbuilt automatic fan and heating system to manage temperature. The temperature ranged from 13°C to 37 °C with a mean ambient air temperature of 21.9 °C (temperature probe with internal sensor wrapped in 35 mm plastic case, Energy Engineering Ltd., Auckland, New Zealand).

Figure 4.1 Pak Choi seedlings (*Brassica rapa* subsp. *chinensis*) grown in the glasshouse

4.2.2 Source of vermicomposts

A total of eleven types of different vermicomposts were used in a range of pot experiments. Three groups of vermicomposts which came from different sources were used in the experiment, and were described below.

The first group of vermicomposts were derived from Vermicomposting Experiment 1 (Chapter 3). At the end of that experiment, twelve vermicomposts were harvested and identified by their composition of initial earthworm feeding materials. Six types of these vermicomposts were further used in The Pot Experiment 2 (for details and the physico-chemical characteristics of these vermicomposts refer to previous chapter: Table 3.3)

The second vermicompost was produced from earthworms raised in a vermicompost bin, which had been fed on fruit and vegetable wastes. It was produced using a similar way to that produced by an domestic household composter. This vermicompost was used as a referance material for comparison with the waste generated vermicomposts.

The final group of vermicomposts were produced from a prepartory experiment which was set up to investigate how a dairy industry waste—used animal bedding (UAB) —can affect vermicompost quality. The experiment was established in an ambient temperature room which protected earthworms from wind and strong sunlight, located at the horticultural nursery at Lincoln University. A mixed age population of earthworms (0.5kg) was raised in a double layer commercial earthworm bin (55cm×30cm×18cm; 30L). Four different substrates were added to the bins as earthworm feedstock, which consist of 1: Fresh shredded waste (FS) 2: Fresh shredded waste + 20% used animal bedding (FS + 20% UAB) 3: Fresh Shredded waste + 20% sawdust (Similar to the initial material of UAB) (FS + 20% S); 4: Fresh shredded waste + cow slurry (FS + CS) (with equivalent amount in UAB). Earthworms were fed regularly every five days at a feeding rate at 200g feeding mixture/day (based on dry mass). The moisture content during the experimental period was maintained at 70±5% by periodic sprinkling the waste with water. There were six replicates for each treatment. After 90 days, the vermicompost was harvested from the bottom layer of each earthworm bin, and sieved (2mm) to remove earthworms and undigested waste. The harvested homogeneous vermicompost was analysed for future use in the pot experiments.

Physico-chemical characteristics and nutrient status of the four types of vermicompost are listed below (Table 4.1):

Table 4.1 Physico-chemical characteristics and nutrient status of vermicomposts

	Analysis	FS	FS + 20% UAB	FS + 20% S	FS + CS
	pH	6.35 ± 0.03	5.84 ± 0.01	6.04 ± 0.02	6.22 ±0.04
	EC (mS/cm)	3.79 ± 0.03	4.64 ± 0.21	3.67 ± 0.16	4.69 ± 0.08
	Total Carbon (%)	14.30 ± 0.47	18.90 ± 0.35	15.43 ± 0.75	15.80 ± 0.18
	Total Nitrogen (%)	1.17 ± 0.04	1.38 ± 0.04	1.14 ± 0.06	1.26 ± 0.01
	Ammonium-N (µg/g)	30.51 ± 1.78	95.87 ± 14.26	27.33 ± 5.52	39.69 ± 0.93
	Nitrate-N (µg/g)	348.6 ± 30.31	95.10 ± 16.24	324.46 ± 106.99	260.54 ± 18.25
	N/C Ratio	12.21 ± 0.11	13.72 ± 0.20	13.53 ± 0.10	12.52 ± 0.12
Water Extractable Plant Nutrients (ppm)	**P**	28.95 ± 4.40	60.59 ± 0.39	33.23 ± 2.15	25.84 ± 0.48
	K	229.87 ±19.85	264.13 ± 12.85	190.33 ± 2.92	271.64 ± 3.07
	Mg	169.91 ± 4.16	193.22 ± 2.09	153.88 ± 6.26	175.17 ± 3.87
	Na	87.90 ± 0.52	90.50 ± 0.36	79.50 ± 2.56	86.10 ± 0.79
	S	14.94 ± 1.28	24.52 ± 0.33	11.66 ± 0.67	13.69 ± 0.78
	Mn	2.02 ± 0.08	1.20 ± 0.02	0.48 ± 0.03	0.95 ± 0.01
	Zn	0.18 ± 0.02	0.33 ± 0.10	0.17 ± 0.02	0.13 ± 0.01
	Fe	0.33 ± 0.14	0.25 ± 0.20	0.05 ± 0.01	0.11 ± 0.02
	Al	0.63 ± 0.28	0.50 ± 0.36	0.10 ± 0.01	0.19 ± 0.03

* Key to Table 4.1: FS: Vermicompost derived from fresh shredded waste; FS + 20% UAB: Vermicompost derived from fresh shredded waste + 20% used animal bedding; FS + 20% S: Vermicompost derived from fresh Shredded waste + 20% sawdust; FS + CS: Vermicompost derived from fresh shredded waste + cow slurry.

4.2.3 Plant selection and potting media

The plant species used in all pot experiments was Pak Choi (*Brassica rapa* subsp. *Chinensis* cv. Mini Leaf). Seeds were obtained from Kings Seeds Company Ltd., Katikati, New Zealand. The species was selected due to its simple structure and fast growth.

There were four potting media used in the experiments were coir, peat moss, sand, vermiculite and a standard mixed potting medium. Additionally, a standard seed-starting mix was also used to raise seedlings for Pot Experiment 5 and 6.

The primary medium used was coir, which is a dark brown, fibrous, peat-like material. It was selected due to the recent interest in using coir for soil-less horticulture production. There is also a lack of study relating to combine vermicompost and coir together as potting medium (Bagci et al., 2011).

The coir and coir-based medium were produced by a compressed coir block from Tumbleweed Pty Ltd, 14 Williamson Road, Ingleburn. These blocks, as they had been desalted, are good to use for horticultural purposes. Before use, the blocks were soaked in tap water at a ratio of 1:5 (coir block: water) for about 30 mins.

The peat moss used was sphagnum peat moss purchase from KIWI PEAT®, Central Otago, New Zealand. It is a dead fibrous material made by decomposed mosses and associated living materials. Due to its good water retention, it is commonly used as a major component in most of potting substrate (Bagci et al., 2011). The vermiculite (grade 2: 1-3mm) used in Pot Experiment 1 was manufactured by Exfollators Ltd, 3 Kitchen Road, Dandenong. This is an expandable 2:1 mineral and often forms from alteration of mica, and has been reported as the most common physical growth substrate in horticultural production and scientific study because of its high water holding, inert chemical nature, moderate level of aeration, absence of substrate for microbial growth and effective cation-exchange capacities (Indrasumunar & Gresshoff, 2013). Horticultural silicon sand, consisting with 80% of coarse sand (0.25-2.00mm) and 20% small gravel (>2.00mm), was purchase from a local garden center.

A standard potting mix (3-4 months) was used as a control medium. This mix was conposed by 80% composted bark, 20% pumice (grade 1-7), 3 kg/m^3 Osmocote® Extract (16-3.5-10), 1 kg/ m^3 horticulture lime and 1kg/ m^3 Hydraflo (a wetting agent). The standard potting mix fertilisers minus fertilisers (3 kg/m^3 Osmocote® Extract (16-3.5-10), 1 kg/ m^3 horticulture lime) was also used in the experiments in the chapter.

The standard seed-starting mix, used for raising seedling in the experiments 5 and 6, was contented 60% barked peat, 40% sterilised pumice, 2 kg/m^3 Osmocate® extract mini, 4kg/m^3 dolomite lime, and 1kg/m^3 Hydraflo.

4.2.4 Chemical analyses

4.2.4.1 Analysis of vermicompost trace elements

Macronutrients and trace elements (P, K, Mg, Na, S, Fe, Mn, Al and Zn) in the vermicomposts were determined using calcium nitrate ($Ca(NO_3)_2$) extraction (Simmler et al., 2013).

For the analysis, air dried samples (5g) and 30 ml 0.05M $Ca(NO_3)_2$ were placed in a 50 ml centrifuge tube, and mixed well using a vortex mixer, and then shaken on the end-over-end shaker for 2 hours. Thereafter, samples were centrifuged at 10000 rpm for 10 minutes and filtered with Whatman No. 52 into 30 ml vials before analysed by ICP-OES (Varian 702-ES).

4.2.4.2 Analysis of Chlorophyll content

Chlorophyll content was determined using 80% acetone as the solvent. A ratio of 0.1g/10ml acetone was mixed together and stored in a dark condition for 5 days to measure chlorophyll a, chlorophyll b and carotenoid content (Jeoung et al., 2013). These were determined using a UV 160A spectrophotometer (Shimadzu, Japan) at different wavelengths of 663, 645, and 470mm, respectively. The final values of chlorophyll a, chlorophyll b and carotenoid were calculated using the formulae:

$$\text{Chlorophyll a} = 12.7\ A663 - 2.69\ A645$$

$$\text{Chlorophyll b} = 22.9\ A645 - 4.68\ A663$$

$$\text{Total chlorophyll (a + b)} = 20.29\ A645 + 8.02\ A663$$

$$\text{Total carotenoid} = (1000\ A470 - 1.82\ \text{chl. a} - 85.02\ \text{chl. b}) / 198$$

* A = wavelength

4.2.4.3 Other chemical analyses

Other chemical analyses used such as pH, electricity conductivity, total carbon and nitrogen and ammonium-N ($NH4^{+}$-N) and nitrate-N ($NO3^{-}$-N) were described in 3.2.3.

4.2.5 Vegetative growth parameters

4.2.5.1 Plant mass

Fresh weight of leaves, shoots and roots

The fresh weight of a plant or its parts was measured immediately after harvest in order to minimise water loss. For the fresh weight of top parts of plants such as leaves or shoots, obvious dust or surface moisture was removed before weighing. The roots were washed and gently blotted using towel tissue and were left at room temperature for about four hours before weighing.

Dry weight of leaves, shoots and roots

The dry weight of plant leaves, shoots and roots was measured after determining their fresh weight. Harvested plants or its parts were labelled and dried in a forced-draft oven dryer at 65°C for 24h. Samples were then placed in a dry environment for about 10-15 minutes to cool before weighing.

4.2.5.2 Plant height

Root length

For root length, dried samples were used to avoid possible influences from post-harvest water loss.

At the end of every experiment, harvested roots were removed and washed with tap water to remove potting substrate. Roots were then air dried, labelled, stored in paper bags and place in a forced-draft oven at 65°C for 24h. Dried samples were thereafter measured from the first node to the end of roots.

Shoot length

The shoot length was also measured using dried plant materials from the apex of the main stem to the base of the plant.

4.3.5.3 Plant development

Number of leaves

The leaf number were calculated by counting the number of emerged leaves excluding cotyledons.

Leaf area and leaf area ratio

The leaf area was calculated using the method of Pandey and Singh (2011). Leaves were cut off and spread over millimeter graph paper to draw a out line of leaves. The outline of leaves were then cut and weighted. In addition, 1 cm^2 of the same millimeter graph paper was also weighted for ten times to calculate the average weight of the graph paper per cm^2. The leaf area can be calculated by using the equation:

$$\text{Leaf area } (cm^2) = x/y$$

* x is the weight of graph paper covered by the leaf outline (g); y is the weight (g) of the cm^2 area of the graph paper.

Leaf area ratio was measured using the formula below according to Lambers et al. (2008).

$$\text{Leaf area ratio (LAR)} = A/W$$

* A is the leaf area; W is the dry mass of leaves.

Root/shoot ratio

The root shoot ratio was calculated based on dried material using the formula:

$$\text{Root/shoot ratio (R/S ratio)} = \text{Dry weight of roots/ Dry weight of shoots}$$

Germination time, germination rate and plant survival rate

Germination time was recorded as the days from planting to the emergence of the first cotyledon.

Germination rate is presented as the percentage of seeds that germinated in the experimental conditions. It was calculated by using the formula below:

$$\text{Germination rate (\%)} = \text{Number of germinated seeds/ Total number of planted seeds}$$

*The number of germinated seeds is counted by the number of seeds with the emergence of the first cotyledon.

Plant survival rate was calculated as the ratio of the number of germinated plants and the total plant number at the end of experiments.

4.2.6 Statistical analysis

Data from the experiment were processed and analysed using JMP (SAS) assisted with Excel (Microsoft 2011). One way Analysis of Variance (ANOVA) was used to determine the significant difference of treatments in all pot experiments on various plant growth parameters, nutrient content of vermicompost and chlorophyll content. Post-hoc tests, Tukey's HSD test (Pot Experiment 1, 2) and Student's t-test (Pot Experiment 3-6) were used to determine the significance among different treatments for various growth parameters (e.g. plant dry weight, shoot length, root length, leaf area, chlorophyll content).

4.3 Pot Experiment 1: Effects of vermicompost in different growth media on Pak Choi seed germination and growth

4.3.1 Treatments and experimental design

The first pot experiment was conducted to investigate whether plant growth medium can affect the performance of vermicompost on plant growth. The experiment ran for two weeks from February 04 to February 18. During this period, the temperature in the greenhouse fluctuated from 14°C to 35°C, with a mean of 23°C.

Plant growth media used in the experiment were coir, peat moss, horticultural silicon sand and vermiculture. Eight treatments were applied, based on the type of growth medium and vermicompost application (four control treatments of each medium and four treatments with 5% vermicompost addition). The vermicompost used was the FW-based (fruit and vegetable waste) vermicompost as described in 4.2.2. The containers used were seed raising trays (37cm×23cm×5cm), which were commonly used for seed breeding. Pak Choi seeds (40) were sown in each tray to mimic standarded seed raising conditions in greenhouse and to evaluate the effect of vermicompost on seed growth.

4.3.2 Experimental procedure and assessments

Eight seed raising trays were filled with 3 litre of eight types of media (eight treatments as mentioned above). Uniform seeds were selected and sown in each medium and lightly covered. These trays were then placed in the greenhouse and watered daily with 320 ml water using a fine mist sprayer. The number of emerged seedlings was recorded daily during the first week. The seedlings were harvested after 14 days to measure plant growth parameters including shoot length, root length and plant dry weight. The number of plants in each tray was also counted at the end of the experiment.

4.3.3 Results and discussion

Generally, vermicompost application resulted in a growth promoting effect on various growth parameters for all tested media throughout the experimental period. Differences between vermicompost treatments and controls were noted just after the emergence of seedlings by looking at the size of cotyledons (Figure 4.2). These differences then became more obvious at the end of the experiment (Figure 4.3). However, it seemed that vermicompost application did not affect emergence of Pak Choi seedlings. In addition, it was noted that Pak Choi seedlings grown in vermiculite amended with vermicompost had darker leaves, while plants in peat moss had pale yellow leaves with dried leaf edge.

In terms of plant dry weight, seedlings grown in peat moss alone had the highest aveage plant dry weight followed by sand, vermiculite, and coir. However, with 5% vermicompost addition, the highest dry weight was found for the seedlings grown in vermiculite followed by coir, sand and peat moss (Table 4.2). Due to the light weight of pak choi seedlings, the significances of plant dry weight was not tested in the experiment. Seedling grown in a medium with 5% vermicompost addition had a significantly higher shoot length and root length than its control, no matter what type of medium used.

It is also noticeable that the differences in dry weight production from the different media were quite high with the highest dry weight at 56.1mg (vermiculite) and the lowest at 29.2mg (peat moss). These variables may be explained by differences in the initial nutrient content and physical structure of the media. An earlier study has found that coir contains high amounts of phosphorus and potassium due to the physiological characteristics of the coconut plant from which is produced (Bagci et al., 2011). Research also showed that significant quantities of mineral nitrogen (N) can be released from vermiculite under warm and moist conditions although it is commonly misused as a nitrogen- free medium (Indrasumunar & Gresshoff, 2013). Thus, the darker colour and greater dry weight of Pak Choi seedling grown in vermiculite may have resulted from the additional N released from the vermiculite. It is not known why plants grown in peat moss had yellow leaves and dried leaf edge. Nevertheless, these signs look like visual symptoms of plant nutrient deficiencies, probably due to the lower pH of peat moss (Bagci et al., 2011).

The shoot and root length in different treatments are shown in Figure 4.4 and Figure 4.5. It can be seen from the Figures, the best medium for the development of the shoot and root system with vermicompost application was vermiculite, with an average shoot and root length at about 2.69 cm and 10.10 cm, respectively (Table 4.2). Vermiculite also had the highest vermicompost use efficiency (yield per unit vermicompost input) with a 48.1mg increase in dry matter weight under 5% vermicompost application, followed by coir (35.4 mg), sand (31.9mg), and peat moss (14.2mg). Peat moss was not an effective medium for raising Pak Choi seedling, either alone or when combined with vermicompost since the seedlings produced were very weak (higher shoot and root length, but with significantly lower day mass) (Table 4.2). Sand is also not a suitable material to combine with vermicompost, at least under a lower percent of vermicompost application rate, as the poor water retention of sand-based medium resulted in non-uniform plant growth along with lower plant survival rate. For coir, although the plant growth parameters were in a moderate range, it is still a suitable medium for horticultural production when combined with vermicompost, and unlike others, it is a renewable resource.

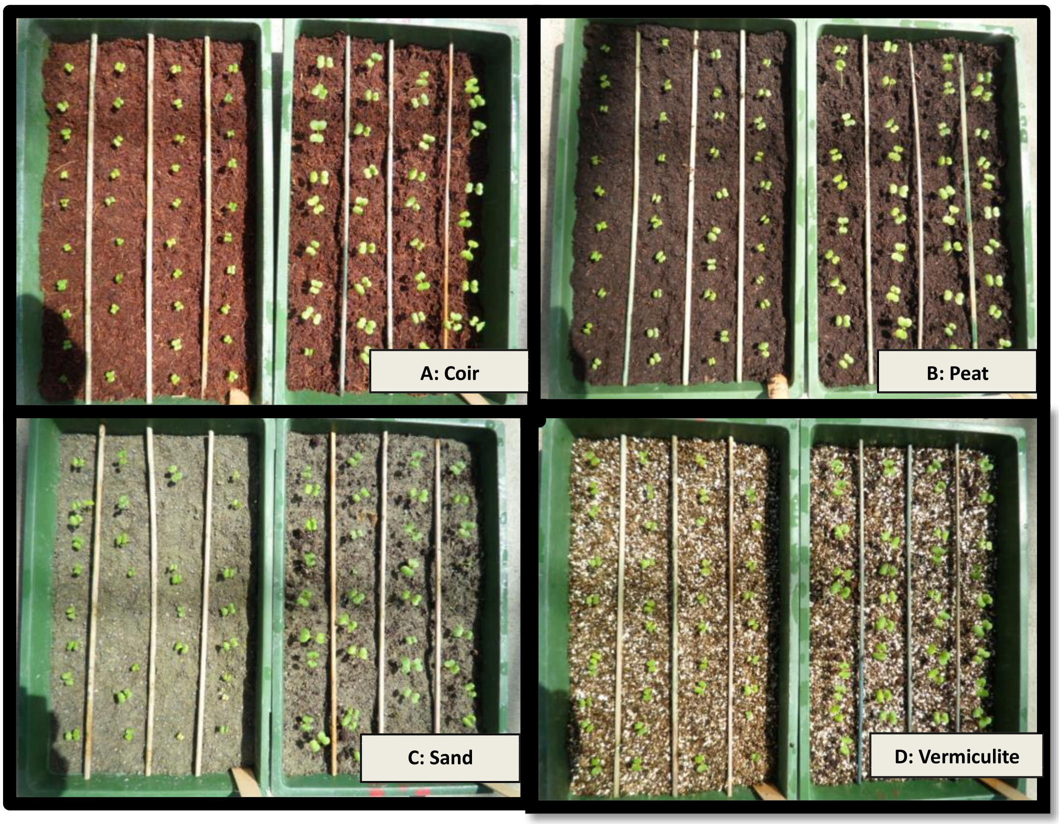

Figure 4.2 Growth of Pak Choi seedlings in the four types of media after three day

* A: Pak Choi grown in coir without (left) and with (right) 5% vermicompost addition;
B: Pak Choi grown in peat without (left) and with (right) 5% vermicompost addition;
C: Pak Choi grown in sand without (left) and with (right) 5% vermicompost addition;
D: Pak Choi grown in vermiculite without (left) and with (right) 5% vermicompost addition.

Figure 4.3 Growth of Pak Choi seedlings in the four types of media after two weeks

* A: Pak Choi grown in coir without (left) and with (right) 5% vermicompost addition;
B: Pak Choi grown in peat without (left) and with (right) 5% vermicompost addition;
C: Pak Choi grown in sand without (left) and with (right) 5% vermicompost addition;
D: Pak Choi grown in vermiculite without (left) and with (right) 5% vermicompost addition.

Table 4.2 Growth parameters of Pak Choi seedlings grown in different media with or without vermicompost addition

	Medium	Number of Survived Plants	Shoot Length (cm)	Root Length (cm)	Average Dry Weight (mg)
Control	**Coir**	40	1.10 ± 0.25 d	4.48 ± 0.69 de	5.2
	Peat	40	1.79 ± 0.38 bc	3.56 ± 0.85 e	10.3
	Sand	33	1.61 ± 0.38 c	3.40 ± 0.96 e	15.0
	Vermiculite	38	1.16 ± 0.33 d	5.99 ± 1.99 bc	8.0
Vermicompos	**Coir**	35	2.44 ± 0.56 a	7.18 ± 1.96 b	40.6
	Peat	40	2.72 ± 0.46 a	5.42 ± 1.66 cd	29.2
	Sand	36	2.05 ± 0.50 b	4.82 ± 1.28 cd	46.9
	Vermiculite	37	2.69 ± 0.69 a	10.10 ± 3.10 a	56.1

* All values are in Means ± Std Deviations. Number of replicates (n) depends on the number of survived plants.

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD test), and indicated by different letters.

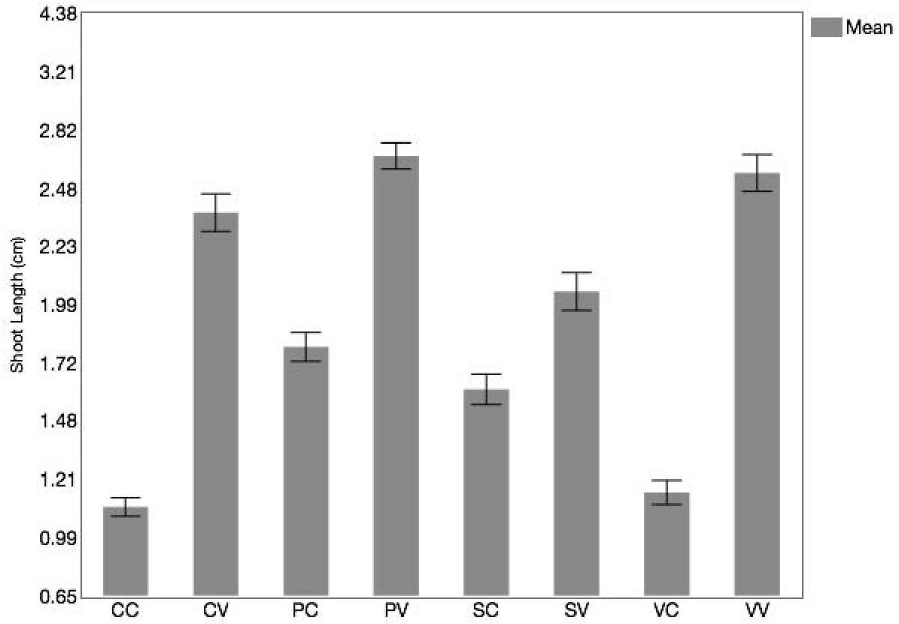

Figure 4.4 Mean shoot length of Pak Choi seedlings grown in various growth media

* CC: A control medium of coir (no other amendments); PC: A control medium of peat moss ; SC: A control medium of horticultural sand; VC: A control medium of vermiculite; CV: Medium contains coir and 5% vermicompost; PV: Medium contains peat and 5% vermicompost; SV: Medium contains sand and 5% vermicompost; VV: Medium contains vermiculite and 5% vermicompost.

* Each error bar is constructed using standard error from the mean.

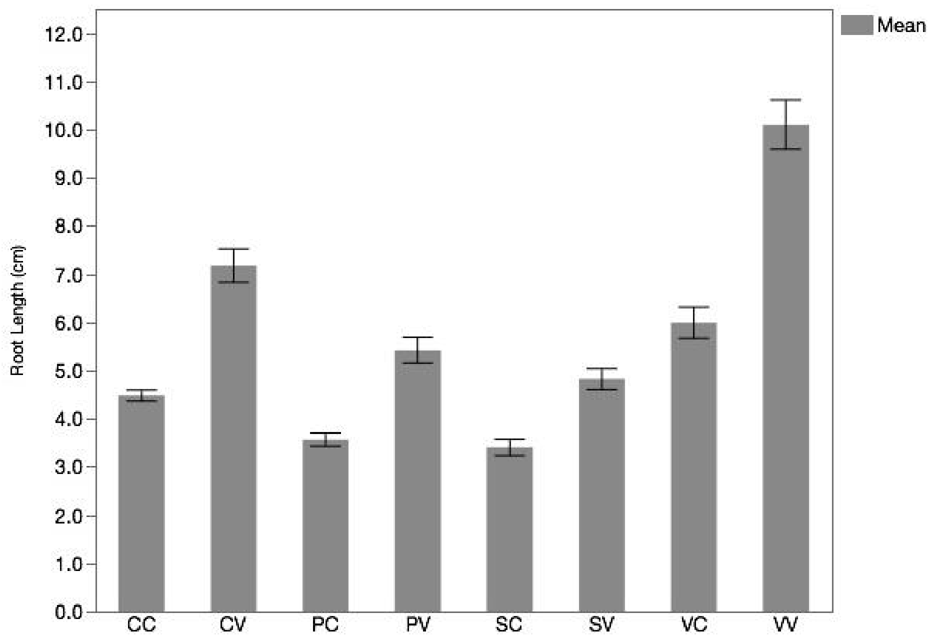

Figure 4.5 Mean root length of Pak Choi seedlings grown in various growth media

* CC: A control medium of coir (no other amendments); PC: A control medium of peat moss; SC: A control medium of horticultural sand; VC: A control medium of vermiculite; CV: Medium contains coir and 5% vermicompost; PV: Medium contains peat and 5% vermicompost; SV: Medium contains sand and 5% vermicompost; VV: Medium contains vermiculite and 5% vermicompost.

* Each error bar is constructed using standard error from the mean.

4.4 Pot Experiment 2: Effects on Pak Choi seedling growth using vermicompost derived from different sources

4.4.1 Treatments and experimental design

The second pot experiment was used to test whether there were differences in agronomic value of the vermicomposts produced in the Vermicomposting Experiment 1. This experiment was conducted in a greenhouse for two weeks from 19 February to 03 March. The temperature, during the experimental period, fluctuated from 15°C to 37°C with a mean temperature of 23.1°C.

There were totally seven types of vermicomposts used in this experiment. Six of them were collected at the end of the Vermicomposting Experiment 1 (Chapter 3). These vermicomposts were originally from three types of municipal organic wastes (Fresh shredded waste (FS), hot rotted waste (HR) and old shredded waste (OS)) mixed with four levels (0 %, 5%, 10% 20%) of a dairy-shed waste, used animal bedding (UAB). However, because FS was found to have advantages over other types of wastes, four FS-based vermicomposts (FS, FS + 5% UAB, FS + 10% UAB, FS + 20%UAB) with two controls of OS and HR generated vermicomposts were used in this experiment. A vermicompost derived from fruit and vegetable waste was also used as a second control. The potting medium used was coir and a standard potting mix (for details see 4.2.3). There were a total of 16 treatments in this experiment (two media amended with seven types of vermicompost plus two controls of each medium without vermicompost amendment). The vermicompost application rate was 5%. The seed raising trays as described in Pot Experiment 1 were also used with 40 seeds sown per tray.

4.4.2 Experimental procedure and assessments

Uniform seeds were selected and sown in the trays filled with different vermicomposts and medium as described above. The trays were then placed in the greenhouse and watered daily during the experimental period. Vegetative growth parameters such as root length, shoot length, plant dry weight were analysed at the end of the experiment.

4.4.3 Results and discussion

In the coir-based medium, municipal and dairy industry waste derived vermicomposts had no obvious effect on plant shoot and root length at an application rate at 5% (Figure 4.6 and Table 4.3). However, a slight increase in plant dry weight was found for all vermicompost treatments, especially for the treatment amended with CF4 (vermicompost derived from fresh shredded waste mixed with 20% used animal bedding) (Table 4.3). FW (Vermicompost derived from fruit and vegetable waste) addition resulted in a great improvement in plant dry weight with about 40.36mg compared to the control (5.24mg). The seedlings grown in coir amended with 5% FW vermicompost performed as well as the seedlings grown in the standard potting mix in terms of shoot length and root length (Figure 4.7, Figure 4.8). The great growth promoting effects of FW vermicompost may have been due to its significantly higher content of NH_4-N and NO_3-N, with containing more than twenty times plant available nitrogen than the domestic waste-derived vermicomposts (Figure 4.9).

For the treatments based on standard potting mix, the vermicompost applications generally led to an increase in seedling dry weight of about 38% (20mg) compared with standard potting mix without vermicompost amendment (Table 4.3). However, it was noticeable that plants growing in the mix amended with OS (vermicompost generated from old shredded waste) had much shorter shoots than control (1.90cm compared to 3.27cm) despite there being no changes in the plant dry weight. These differences may have been due to the higher soluble salt content (higher electricity conductivity) of the OS vermicompost. Interestingly, There was no much difference between FW vermicompost and the domestic waste generated vermicompost in terms of average dry weight, shoot and root length, although FW vermicompost has significantly higher NH_4-N and NO_3-N content. It was likely because the amount of chemical fertiliser in the potting mix had been sufficient, nitrogen source was no longer the primary factor that limited plant growth. Thus, the extra N inputs from FW vermicompost did not affect plant growth since fertiliser applications generally follow the law of diminishing returns (Tittarelli et al., 2007). It could be speculated that the growth promoting effects of vermicompost when combined with standard potting mix may be mainly due to the physical or biological properties of vermicompost rather than its nutrient content.

Figure 4.6 Pak Choi seedlings amended with different types of vermicompost grown in the greenhouse

* First row: Seedlings grown in coir amended with 5% vermicompost derived from different sources;
Second row: Seedlings grown in coir amended with 5% vermicompost derived from different sources.

Table 4.3 Growth parameters of Pak Choi seedlings grown in different media amended with different types of vermicompost

Treatment	Number of Survived Plants	Shoot Length (cm)	Root Length (cm)	Average Dry Weight (mg)
CFW	39	3.28 ± 0.59 a	7.76 ± 2.40 ab	45.6
CC	39	1.63 ± 0.38 b	5.28 ± 1.39 d	5.24
COS	40	1.90 ± 0.43 b	6.12 ± 2.29 bcd	5.42
CHR	40	1.71 ± 0.46 b	6.07 ± 2.16 cd	5.88
CFS	39	1.78 ± 0.41 b	5.38 ± 1.95 d	5.77
CFS2	38	1.66 ± 0.39 b	6.09 ± 2.18 bcd	5.87
CFS3	38	1.71 ± 0.50 b	6.17 ± 2.02 bcd	5.42
CFS4	39	1.74 ± 0.38 b	6.27 ± 1.86 bcd	6.32
PFW	40	3.41 ± 0.69 a	6.75 ± 2.04 bcd	73.04
PC	40	3.27 ± 0.62 a	6.45 ± 1.61 bcd	51.75
POS	40	1.90 ± 0.43 b	6.10 ± 2.29 cd	75.27
PHR	37	3.38 ± 0.77 a	6.95 ± 2.25 bcd	74.70
PFS	40	3.50 ± 0.58 a	7.49 ± 2.00 abc	75.74
PFS2	40	3.516 ± 0.85 a	6.68 ± 1.99 bcd	70.11
PFS3	39	3.356 ± 0.52 a	8.86 ± 3.15 a	78.41
PFS4	40	3.462 ± 0.51 a	8.83 ± 2.13 a	73.67

* All values are in Means ± Std Deviations. The number of replicates (n) depends on the number of survived plants.

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Tukey's HSD-test), and indicated by different letters.

* Key to Table 4.3, Figure 4.7 & 4.8: CFW: Coir amended with vermicompost derived from fruit and vegetable waste; CC: Controlled coir medium without vermicompost addition; COS: Coir amended with vermicompost derived from old shredded waste; CHR: Coir amended with vermicompost derived from hot rotted waste; CFS: Coir amended with vermicompost derived from fresh shredded waste. CFS 2: Coir amended with vermicompost derived from fresh shredded waste mixing with 5% used animal bedding. CFS 3: Coir amended with vermicompost derived from fresh shredded waste mixing with 10% used animal bedding. CFS 4: Coir amended with vermicompost derived from fresh shredded waste mixing with 20% used animal bedding. PFW: Standard potting mix amended with vermicompost derived from fruit and vegetable waste; PC: Standard potting mix used as control; POS: Standard potting mix amended with vermicompost derived from old shredded waste; PHR: Standard potting mix with vermicompost derived from hot rotted waste; PFS: Standard potting mix amended with vermicompost derived from fresh shredded waste. PFS 2: Standard potting mix amended with vermicompost derived from fresh shredded waste mixing with 5% used animal bedding. PFS 3: Standard potting mix amended with vermicompost derived from fresh shredded waste mixing with 10% used animal bedding. PFS 4: Standard potting mix amended with vermicompost derived from fresh shredded waste mixing with 20% used animal bedding.

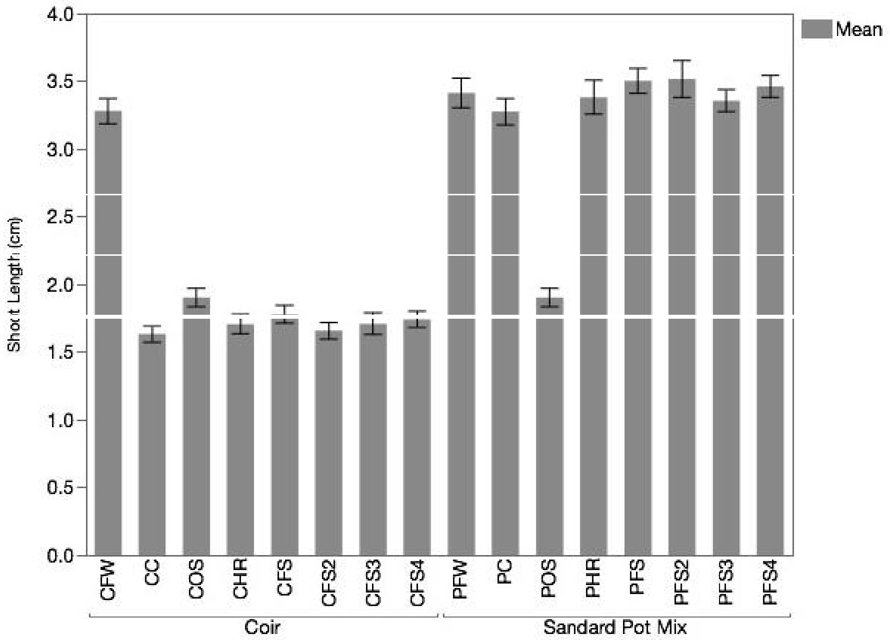

Figure 4.7 Mean shoot length of Pak Choi seedlings grown in coir and standard potting mix amended with different types of vermicompost

* Key as in Table 4.3

* Each error bar is constructed using standard error from the mean.

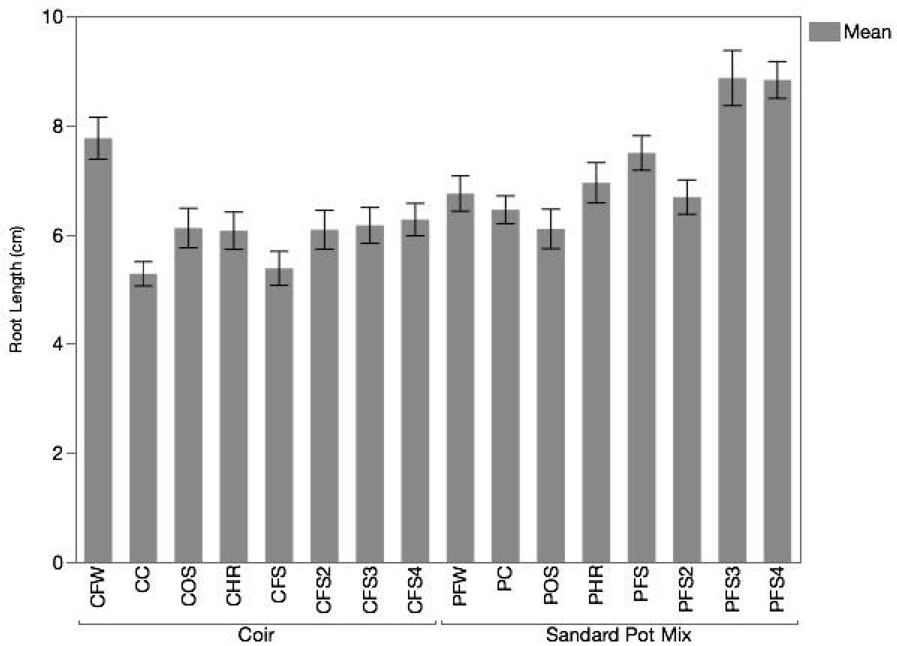

Figure 4.8 Mean root length of Pak Choi seedlings grown in coir and standard potting mix amended with different types of vermicompost

* Key as in Table 4.3

* Each error bar is constructed using standard error from the mean.

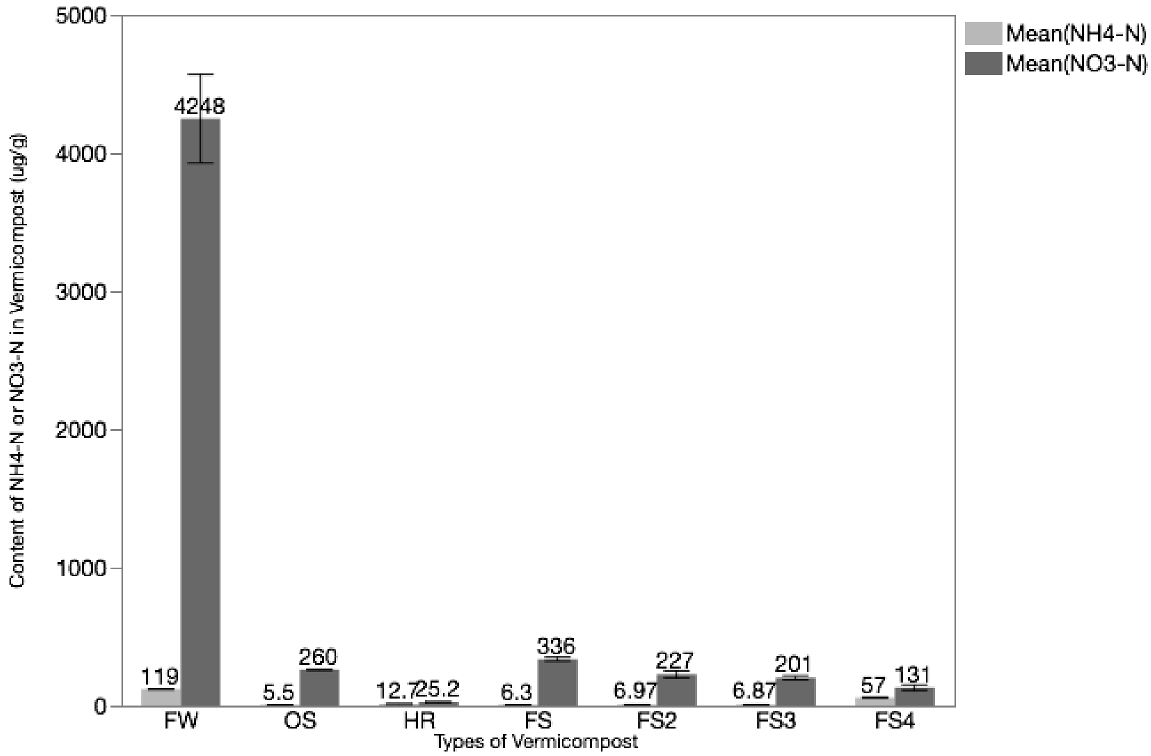

Figure 4.9 Mean ammonium-N and nitrate-N (ug/g) in contents of different vermicomposts

* FW: Vermicompost derived from fruit and vegetable wastes; OS: Vermicompost derived from old shredded wastes; HR: Vermicompost derived from hot rotted wastes: FS: Vermicompost derived from fresh shredded wastes; FS2: Vermicompost derived from fresh shredded waste mixed with 5% used animal bedding; FS3: Vermicompost derived from fresh shredded waste mixed with 10% used animal bedding; FS4: Vermicompost derived from fresh shredded waste mixed with 20% used animal bedding.

* Each error bar is constructed using standard error from the mean.

4.5 Pot Experiment 3: Effect of volume of vermicompost application (concentration) on Pak Choi seedling growth

4.5.1 Treatments and experimental design

The third experiment was designed to investigate how the quantity of vermicompost application affects plant growth. The experiment ran for two weeks from 08 March to 22 March. During the experimental period, the temperature in greenhouse ranged from 14°C to 32°C, with a mean temperature of 20.8 °C. The type of vermicompost used in the experiment was from fresh shredded waste mixed with 20% used animal bedding (the best waste combination according to the previous study) produced from the vermicompost preparatory experiment in the ambient temperature room (for details see 4.2.2). Six-cells seedling trays (4cm×4cm×7cm (per cell)) were used in the experiment, and three media used were coir (C-), standard potting mix (P+) and standard potting mix minus fertilisers (P-). Four levels of vermicompost (5%, 10%, 20%, 30%) were added to each medium respectively with three additional controls of no vermicompost amended medium. Fifteen treatments, each with six replicates were applied, was set up in the same six-cell trays.

4.5.2 Experimental procedure and assessments

First, adequate substrates were made by fully mixing three types of medium with different levels of vermicompost by volume (0%, 5%, 10%, 20% and 30%). After that, 500ml of each prepared substrates were filled in each six-cell seedling trays evenly with every cell contains about 83 ml potting medium. After a slight watering, healthy and uniform Pak Choi seeds were sown in each cell and lightly covered. All trays were then moved and randomly placed on the table in the greenhouse. Plants were watered daily using a measuring cup at a rate of about 10 ml per cell. After 14 days, all Pak Choi seedlings were harvested, and relative plant growth parameters were analysed.

4.5.3 Results and discussion

Figure 4.10 shows Pak Choi seedlings grown in the different media amended with vermicompost in various volumes. It can be seen that there was a major effect of vermicompost concentration on Pak Choi seedling growth for all tested growth medium especially for C- (coir) and P- (Potting mix minus fertilisers) (Figure 4.10: A, B, C). Plants grown in C- (10%) and P- (20%) based media grew as well as the plants grown in standard potting mix (Figure 4.10: D and E).

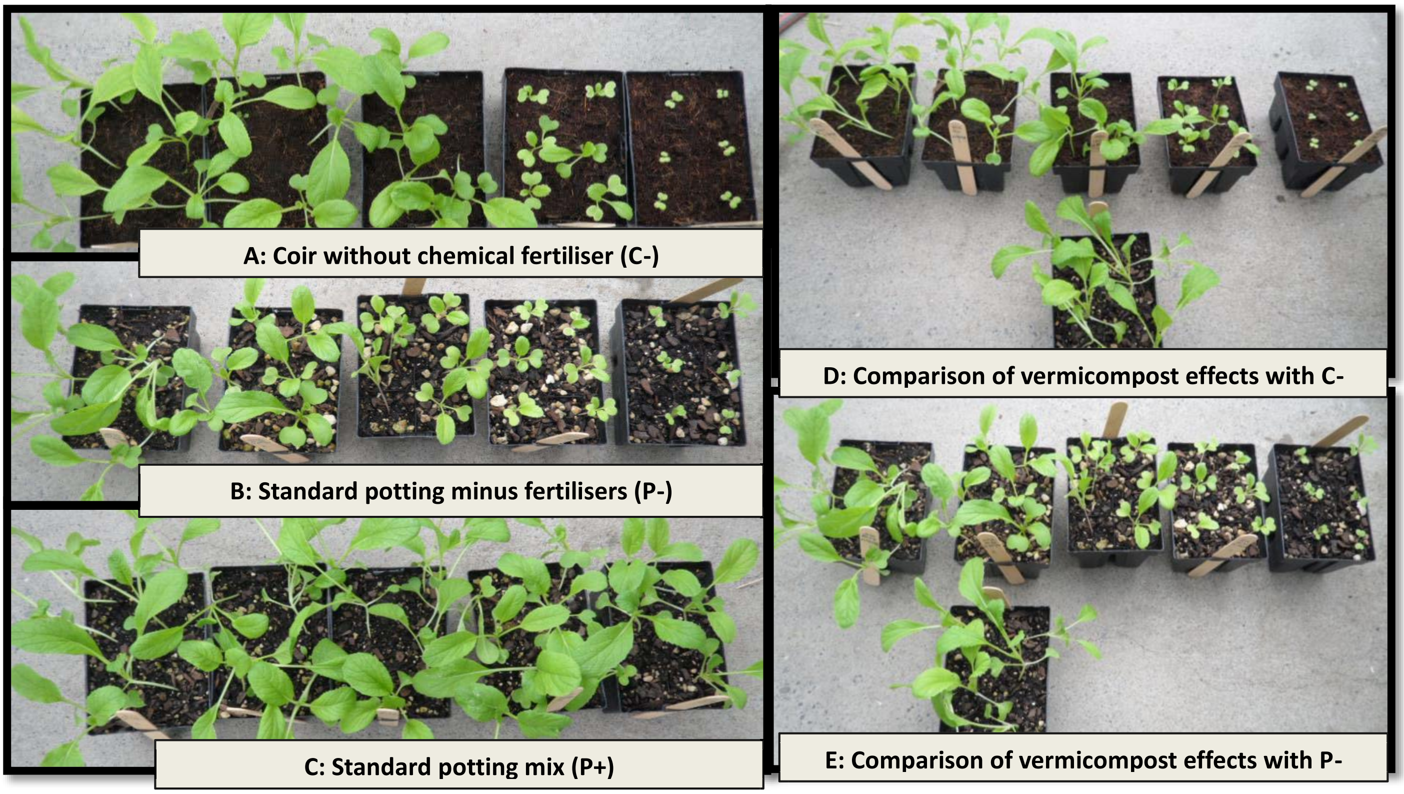

Figure 4.10 Responses of Pak Choi seedlings grown in different media (C-, P-, P+) substituted with vermicompost at various concentrations

* C-: Coir without additional chemical fertiliser; P-: Potting minus fertilisers (80% composted bark + 20% pumice +1kg/ m^3 Hydraflo); P+: A standard potting mix containing 3-4 month plant fertiliser.

* The six-cell seedling trays were ranked in an order of vermicompost concentration from highest to lowest in the photos (from left to right: 30%, 20%, 10%, 5% and 0%).

The effects of the different vermicompost concentration on the growth and development of Pak Choi seedlings could also be shown in various growth parameters (Table 4.4).

Generally, seedlings with more leaves had a higher plant dry weight. The most significant improvement in the number of true leaves was when vermicompost application rate increased from 5% to 10% for C- plants (1.83 to 3.14) and P- plants (1.83 to 2.67), and the average dry weight of these plants increased from 15.63mg to 68.02mg (C-) and 19.65mg to 42.37mg (P-) respectively. There was also a significant improvement in leaf number for P- plants when the concentration of vermicompost was increased from 20% to 30%. However, for P+ plants, no significant differences in leaf numbers found at any rate of vermicompost application. This result suggests that chemical fertiliser contained in P+ medium was adequate for the growth of Pak Choi seedling, and thus, no further improvement has resulted from additional vermicompost.

In terms of shoot and root length, a 10% vermicompost application resulted in the most significant improvement in shoot length for the C- plants at about 46% (2.48cm to 3.63cm) as well as root length at about 56% (8.67cm to 13.53cm) compared to the treatment with the 5% vermicompost application rate. However, there was no significant effects on shoot length for the P- plants when the vermicompost application rate was increased from 5% to 10% (3.20cm to 3.38cm), although a significant improvement in root length was observed (9.98cm to 16.98cm). For p+ plants, there was no significant difference in shoot length and root length under any levels of vermicompost application, apart from a significantly increased root length for 5% application rate (15.93cm).

Table 4.4 Growth parameters of Pak Choi seedlings grown in different media substituted with different concentrations of vermicompost

Treatment	Number of plants	Number of true leaves per plant	Shoot Length (cm)	Root Length (cm)	Average Dry Weight (mg)
CC-	6	0.00 ± 0.00 e	2.02 ± 0.23 d	6.44 ± 1.11 g	4.60
5%C-	6	1.83 ± 0.41 d	2.48 ± 0.28 cd	8.67 ± 2.45 fg	15.63
10%C-	5	3.40 ± 0.55 b	3.63 ± 0.15 ab	13.53 ± 2.52 abcd	68.2
20%C-	6	3.50 ± 0.55 b	3.57 ± 0.36 ab	11.11 ± 1.56 cdef	93.5
30%C-	6	3.83 ± 0.75 ab	2.97 ± 0.42 bc	9.99 ± 2.24 def	100.77
CP-	6	1.33 ± 0.52 d	2.53 ± 0.68 cd	9.68 ±1.86 efg	8.57
5%P-	6	1.83 ± 0.41 d	3.20 ± 0.72 abc	9.98 ± 3.14 def	19.65
10%P-	6	2.67 ± 0.52 c	3.38 ± 0.77 ab	16.28 ± 2.74 ab	42.37
20%P-	6	2.67 ± 0.52 c	3.19 ± 0.71 abc	17.11 ± 5.36 a	62.87
30%P-	6	3.83 ± 0.41 ab	3.40 ± 0.48 ab	14.09 ± 5.04 abc	92.05
CP+	6	3.83 ± 0.75 ab	3.57 ± 0.63 ab	13.22 ± 2.60 bcd	77.75
5%P+	6	4.00 ± 0.63 ab	3.39 ± 0.89 ab	15.93 ± 3.95 ab	118.10
10%P+	6	4.16 ± 0.41 a	3.55 ± 0.61 ab	12.44 ± 2.30 cde	90.85
20%P+	6	4.00 ± 0.63 ab	3.46 ±0.40 ab	11.39 ± 1.99 cdef	99.63
30%P+	6	4.00 ± 0.00 ab	3.87 ± 0.58 a	10.71 ± 2.63 cdef	106.72

* All values are in Means ± Std Deviations. The number of replicates (n) depends on the number of survived plants.

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t-test), and indicated by different letters.

* CC-: Coir as a control; 5%C-: Coir substituted with 5% vermicompost; 10%C-: Coir substituted with 10% vermicompost; 20%C-: Coir substituted with 20% vermicompost; 30%C-: Coir substituted with 30% vermicompost; CP-: Potting mix minus fertilisers; 5%P-: Fertiliser removed potting mix substituted with 5%

vermicompost; 10%P-: Fertiliser removed potting mix substituted with 10% vermicompost; 20%P-: Fertiliser removed potting mix substituted with 20% vermicompost; 30%P-: Fertiliser removed potting mix substituted with 30% vermicompost; CP+: Controlled standard potting mix containing 3-4 month plant fertiliser. 5% CP+: Standard potting mix substituted with 5% vermicompost; 10% CP+: Standard potting mix substituted with 10% vermicompost; 20% CP+: Standard potting mix substituted with 20% vermicompost; 30% CP+: Standard potting mix substituted with 30% vermicompost.

Changes in plant growth parameters with the various concentration of vermicompost are shown in Figure 4.11. The graph A demonstrates how leaf number changed with the concentration of vermicompost. The leaf numbers for the C- and P- plants were both increased with a higher concentration of vermicompost, but this effect did not found for P+ plants. The C- plants, generally, had more leaves than the P- plants under the same vermicompost application rate. The shoot length of Pak Choi plants grown in C- and P- was also increased with the vermicompost concentration, but the shoot length declined when the vermicompost concentration over 10% (Figure 4.11: B). For root length, it seemed that plants grown in the P- and P+ medium generally had higher root length than the plants in C- medium (Figure 4.11: C).

It is noticeable that effects of vermicompost concentration on plant dry weight were affected by the type of medium used (Figure 4.12). Coir (C-) had a high vermicompost used efficiency (biomass per unit vermicompost input) under a low vermicompost applicantion rate, but the growth promoting effect almost ceased when the concentration was over 20%. For P- medium, despite having a lower vermicompost used efficiency, the increased vermicompost concentration had a consistent promoting effect on plant dry weight up to 30%. For P+, as the medium contained sufficient amount of artificial fertiliser, the trend was different from P- and C-. However, a significant increase in plant dry weight was noted when the concentration of vermicompost in the medium was about 5%.

Additionally, visible changes in the structure of Pak Choi seedling was also observed with different vermicompost application rate (Figure 4.13). Plants grown in P- medium were weaker than those grown in coir (longer shoot and root length, but less weight) (Figure 4.13: A). Also, increased amount of vermicompost application resulted in greater leaf area and thicker shoots (greater number of root hair), although the root length was reduced under high vermicompost concentration (Figure 4.13: B).

It is unknown how medium type and vermicompost concentration affect vermicompost use efficiency. However, it may associate with a complex interaction between medium, vermicompost and plants. For example, the shorter root length that occurred (under high vermicompost application rate) may have been due to the higher nutrient concentration in the potting medium, as one of the primary function of plant roots is access to water and nutrients. Thus, these plants could capture adequate nutrients without the need for long roots as explained by the optimal partitioning theory of

McCarthy & Enquist (2007). In addition, the physical properties of a potting medium can also affect the accessibility of roots to nutrients and water.

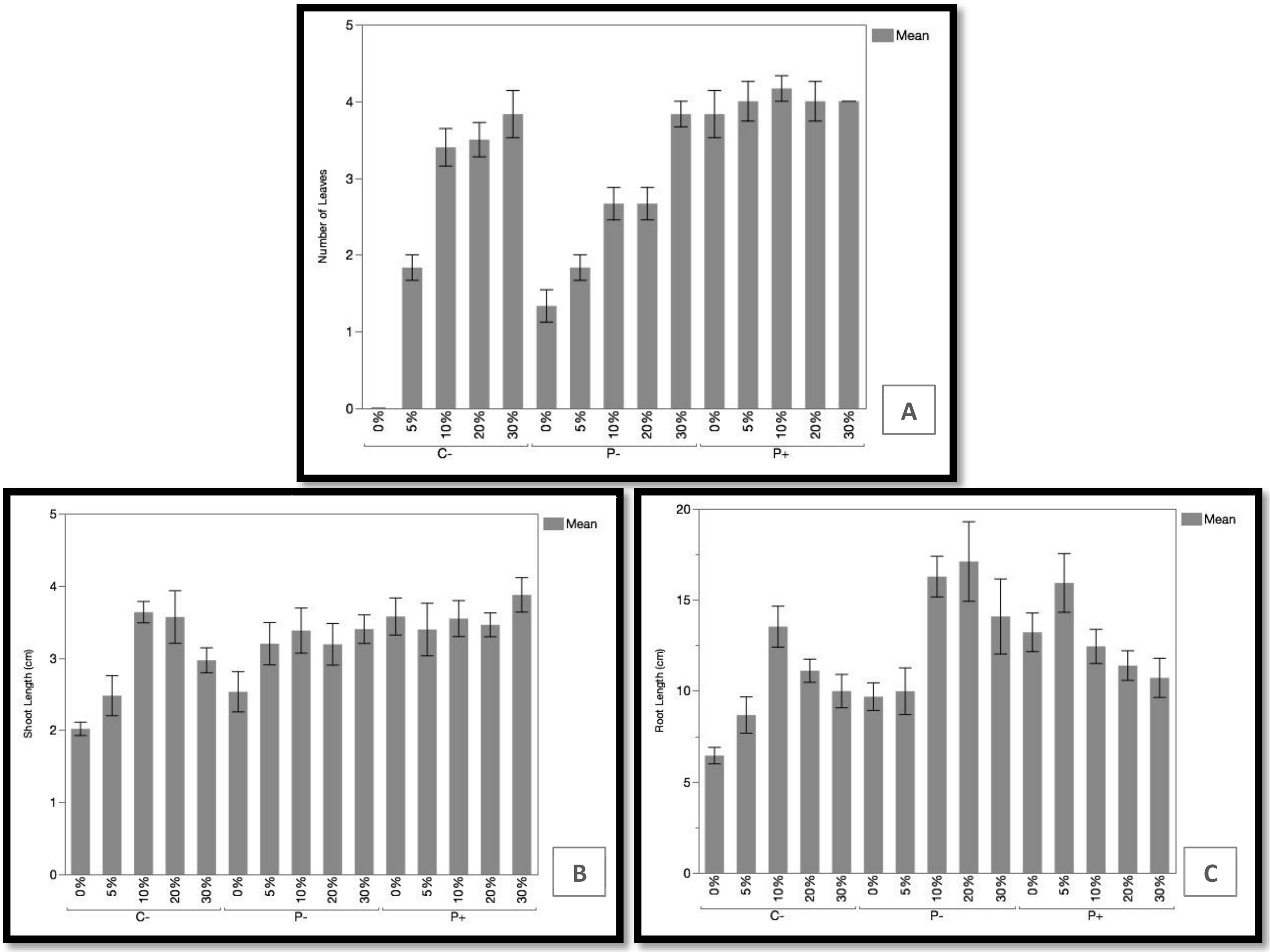

Figure 4.11 Means of leaf number (A), shoot length (B) and root length (C) with various concentration of vermicompost in the three types of potting media

* Key to Figure 4.11: C-: Coir-based medium without additional chemical fertiliser; P-: Potting mix minus fertilisers (80% composted bark + 20% pumice +1kg/ m^3 Hydraflo); P+: A standard potting mix containing 3-4 month plant fertiliser.

* The percentages refer to the concentration of vermicompost in the three types of media by volume.

* Each error bar is constructed using standard error from the mean.

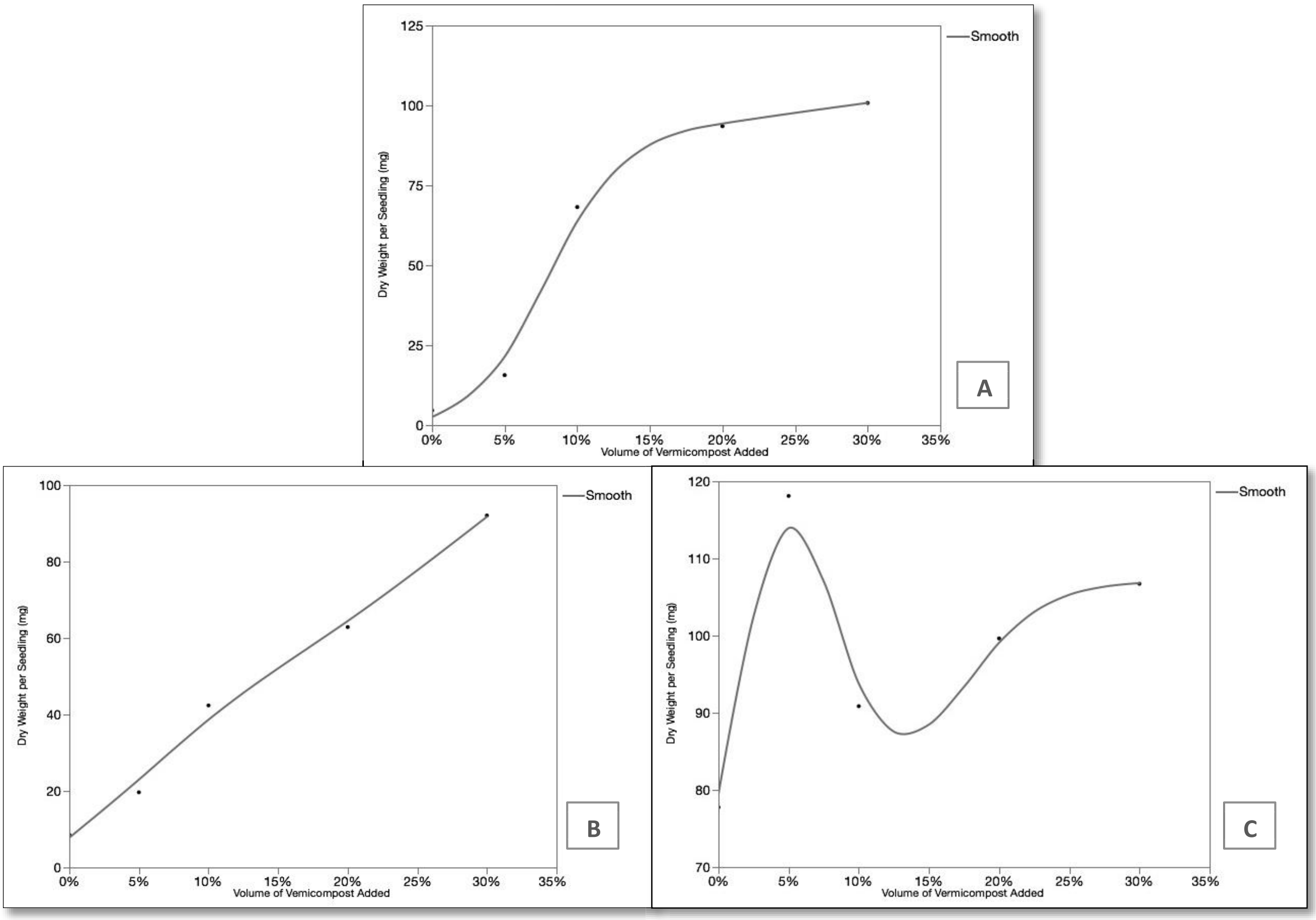

Figure 4.12 Mean average dry weight of Pak Choi seedlings grown in the three types of media with various vermicompost concentrations

* A: Coir (C-) B: Potting mix minus fertilisers (P-) C: Standard potting mix (P+))

Figure 4.13 Pak Choi seedlings grown in different media (A) and under different vermicompost application rates

* Photo A: Pak Choi seedlings grown in P- (left) and C- (right) substituted with 20% vermicompost; Photo B: Pak Choi seedlings grown in C- with different concentration of vermicompost at 0%, 5%, 10%, 20%, 30%, respectively (from left to right).

4.6 Pot Experiment 4: Plant responses to vermicompost additions with different application methods

4.6.1 Treatments and experimental design

The fourth experiment was set up to test how much the methods of application can affect plant responses to vermicompost addition. The experiment ran for two weeks from 08 March to 22 March. During the experimental period, the temperature in greenhouse fluctuated from 14°C to 32°C, with a mean temperature of 20.8 °C.

The materials used in the experiment were the same as in Pot Experiment 3, which were waste-derived vermicompost (FS + 20% UAB) and six-cell seedling trays. The media used were coir (C-) and standard potting mix minus fertilisers (P-). The three application methods tested in the experiment were: 1) mixed with substrates (M); 2) surface application (S); 3) application with watering (W). The vermicompost with an application rate of 10% was applied to the seedling trays containing the two media (C- and P-) using the three different application methods, respectively. Control treatments (C) with no vermicompost amendment were also used for each type of medium. Thus, there was a total of eight treatments in this experiment with six replicates in the same seedling trays.

4.6.2 Experimental procedure and assessments

Eight packs of six-cell seedling trays were filled with the two media (C- and P-) amended with vermicompost applied in different methods (M, S, W and C) according to the design of the experiment (CC-, CP-, MC-, MP-, SC-, SP-, WC- and WP-). Each cell contained about 83 ml substrate including the vermicompost. Uniform Pak Choi seeds were selected and sown in the prepared substrate. For the W treatments, no vermicompost was added at the beginning, but the same amount of vermicompost was mixed with water to produce a 1680 ml vermicompost-water suspension. During the experimental period, each replicate was watered daily with 10 ml water, excluding the W treatments (WC-, WP-) which was watered with the vermicompost-water suspension that had been prepared at the same rate (10ml/day; the suspension was well shaken before application). Records of plant growth were taken daily during the experimental period, and all seedlings were harvested after 14 days for the analysis of vegetative growth parameters including shoot length, root length and average plant dry weight.

4.6.3 Results and discussion

Figure 4.14 shows the differences in plant responses to various vermicompost application methods. The W treatments resulted in a smaller plant size, with the lowest average plant dry weight at 37.03mg and 33.90mg for C- and P- medium, respectively. Plants grown in C- with M treatment resulted in a slightly higher plant dry weight at 58.28mg compared to S treatment at 56.62mg. However, for P-, the greatest improvement in plant dry weight was found with S treatment (62.60mg) compared with M treatment (39.60mg) (Table 4.5). The application methods also had a significant effect on Pak Choi leaf numbers. For C-, M treatment resulted in significantly higher leaf numbers of 3.83 leaves followed by S treatment (3.00) and W treatment (2.33). However, the major effects on leaf number of P- were found with the S treatment with an average leaf number of 3.67, which is also significantly higher than M (2.67) and W treatments (2.17) (Table 4.5; Figure 4.15: A). In general, Pak Choi seedlings had a better growth and development in C- than P- regarding plant dry weight and leaf number.

Shoot and root lengths were generally higher in the P- treatments compared to C- treatments, especially for root length (Figure 4.15: B, C). This result was consistent with the observation in the previous experiment (Pot Experiment 3). Moreover, the highest shoot and root lengths were obtained with M treatment followed by S, W and C, respectively. However, despite M treatments had the highest shoot length, a significantly lower root length with M treatment was also observed for P- in contrast to S and W.

Overall, there has interactions between the type of used medium and vermicompost application methods in this experiment. The results also suggest that vermicompost efficiency (plant yield/biomass per unit vermicompost inputs) can be significantly affected by application methods. It seemed that M treatment was the most efficient application method for C- medium at an application rate at 10%, while the most effective method for P- was S. Although the reason for these results is still unknown, it was probably associated with physical and biochemical differences of potting substrates under different vermicompost application methods.

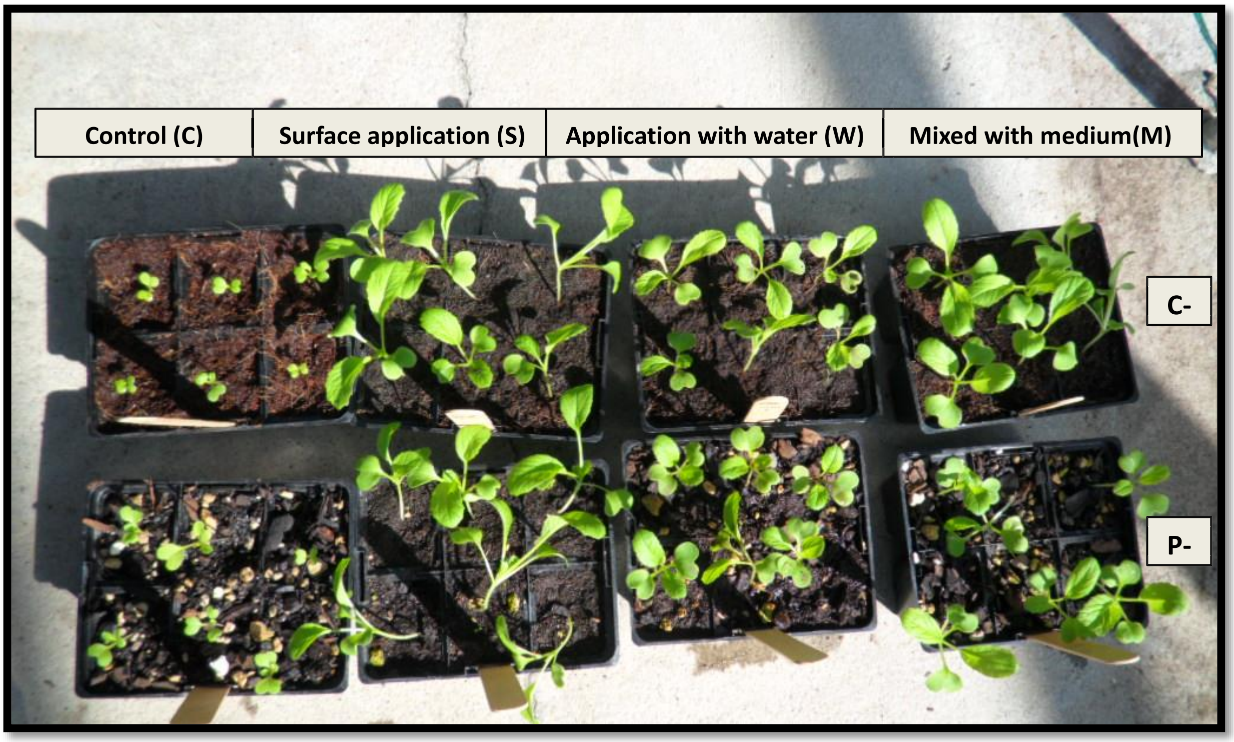

Figure 4.14 Responses of Pak Choi seedlings to different application methods

* The application rate of vermicompost (derived from FS+20%UAB) in the experiment was 10% of total volume of potting substrates.

* C-: Coir-based medium without additional chemical fertiliser; P-: Standard Potting mix minus fertilisers (80% composted bark + 20% pumice +1kg/ m^3 Hydraflo).

Table 4.5 Growth parameters of Pak Choi seedlings with different vermicompost application methods

Treatment	Number of true leaves per plant	Shoot Length (cm)	Root Length (cm)	Average Dry Weight (mg)
CC-	1.00 ± 0.89 f	2.00 ± 0.73 e	7.50 ± 2.04 d	6.25
SC-	3.00 ± 0.89 bc	3.01 ± 0.55 bcd	8.87 ± 1.68 cd	56.62
WC-	2.33 ± 0.52 cde	2.56 ± 0.45 de	8.34 ± 1.47 cd	37.03
MC-	3.83 ± 0.41 a	3.69 ± 0.74 ab	8.86 ± 2.75 cd	58.28
CP-	1.67 ± 0.52 ef	2.75 ± 0.74 cd	9.75 ± 0.76 bcd	8.75
SP-	3.67 ± 0.52 ab	3.19 ± 0.62 abcd	11.99 ±1.95 ab	62.60
WP-	2.17 ± 0.41 de	3.36 ± 0.25 abc	13.91 ± 2.75 a	33.90
MP-	2.67 ± 0.52 cd	3.80 ± 0.67 a	10.52 ±4.01 bc	39.60

* All values are in Means ± Std Deviations (n=6).

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t-test), and indicated by different letters.

* CC-: coir-based medium without additional chemical fertiliser as a control; SC-: 10% of vermicompost applied on the surface of coir medium; WC-: Vermicompost was mixed with water and added to coir medium every day with watering; MC-: 10% vermicompost was premixed with coir before sowing; CP-: Controlled Potting mix minus fertilisers; SP-: 10% of vermicompost applied on the surface of fertiliser removed potting mix; WP-: Vermicompost was mixed with water and added to fertiliser removed potting mix every day with watering; MP-: 10% vermicompost was premixed with fertiliser removed potting mix before sowing.

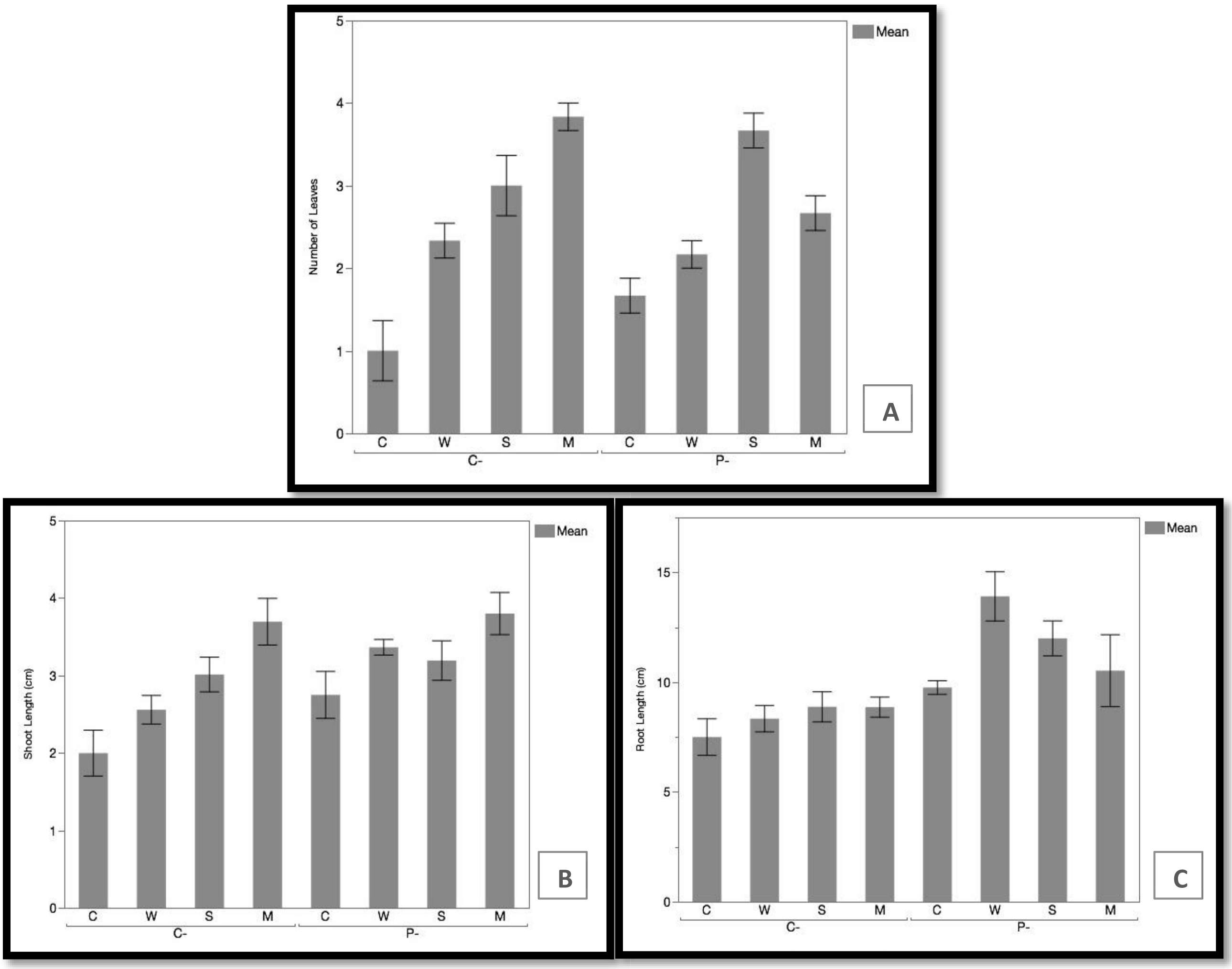

Figure 4.15 Mean leaf number (A), shoot length (B) and root length (C) of Pak Choi seedlings with different vermicompost application methods

* C-: Coir-based medium without additional chemical fertiliser as a control; P-: Potting mix minus fertilisers (80% composted bark + 20% pumice +1kg/ m^3 Hydraflo). C: Control; W: Application with watering; S: surface application; M: mixed with Medium.

* Each error bar is constructed using standard error from the mean.

4.7. Pot Experiment 5: The influence of different vermicompost treatments on Pak Choi growth

4.7.1 Treatments and experimental design

The fifth experiment was conducted to evaluate how much the vermicompost processing method can affect plant growth performance. The greenhouse experiment ran for 20 days from 24 March to 13 April, where the temperature fluctuated from 13°C to 30°C, with a mean temperature of 20.8°C.

The four types of vermicompost used were produced from the vermicompost preparatory experiment in the ambient temperature room, and they were: 1) Fresh shredded waste (FS) 2) Fresh shredded waste + 20% used animal bedding (FS + 20% UAB) 3) Fresh Shredded waste + 20% sawdust (Similar to the initial material of UAB) (FS + 20% S); 4) Fresh shredded waste + cow slurry (FS + CS) (for details see 4.2.2). Three processing methods (F: fresh, OD: oven dried, S: sterilised) were used to treat the four types of vermicomposts. For the fresh treatment (F), each of the four types of vermicompost was stored at room temperature without additional treatment. For the oven dried treatments (OD), vermicomposts were put in a forced-draft drying oven at 65°C for 48 hours. For the sterilised treatment (S), vermicomposts were sterilised in an autoclave (moist heat sterilisation) to kill all microbes. After the processing, all vermicomposts were immediately used in the experiment by mixing with coir as the medium, at a rate of 20%. The containers used were 200ml plastic pots (6cm×6cm×7cm). Control with coir medium without treatments was also applied, and there were six replicates for each treatment.

4.7.2 Experimental procedure and assessments

Four types of harvested vermicomposts (FS, FS + 20% UAB, FS + 20% S, FS + 20% CS) were treated with three different processing methods prior to the experiment. All the treatments were prepared in three days of harvesting.

Before to the experiment begin, adequate number of Pak choi seeds were sown and grown in a standard seed-starting mix (for details see 4.2.3) for raising seedlings used in the experiment. After ten days, uniform seedlings from the trays were selected and transplanted to the plastic pots, which were filled with the pre-mixed coir/vermicompost mixture according to the treatments that have been described above. Established plants were then placed in the greenhouse for a further 20 days, and plants were watered daily at a rate of 30 ml water per pots. At the end of the experiment, 0.5g leaf samples (per treatment) were randomly taken using a 6mm diameter puncher for the analysis of chlorophyll content. Plants were then harvested and measured for growth parameters including leaf area, shoot length, root length and fresh/ dry weight.

4.7.3 Results and discussion

Figure 4.16 and Table 4.6 showed the response of Pak Choi plants to different vermicomposts under the three types of processing method, fresh (F), oven dried, (OD) and sterilised (S). In general, all treatments resulted in significant improvements in plant growth compared to the control, with increases in leaf area about 33 times, and increases in dry weight about 19 times.

For the processing methods, F treatment generally had the least promoting effects on Pak Choi plant growth with fewer leaf number, less leaf area, and lower plant dry weight than OD and S. Additionally, a slightly higher root/ shoot (R/S) ratio, lower total Chlorophyll (Chl.) and carotenoid contents were also observed for F treatment (Table 4.6: part 2). These results suggest that freshly harvested vermicompost may not be suitable to apply directly to plants, and additional processing methods may improve the effects of vermicompost on plant growth. However, it is unknown what are factors leading to the lower development rate of Pak Choi under fresh vermicompost treatments. Nevertheless, the explanation may be associated with the higher microbe population in fresh vermicompost, and nutrient releases from microorganisms in the drying or sterilising process, since microorganisms may compete with plants for available nutrients, and the higher temperature in drying and sterilising process may release nutrients from dead microorganisms.

No significant difference was found between the four types of vermicompost, although the chemical properties vary (Table 4.1). This was probably due to the complexity of vermicompost properties corresponding to the requirements for plant growth. For example, vermicompost derived from FS+20%UAB had a high nutrient status with almost double the content of phosphorus and sulphur compared with other types of vermicompost (4.2.2). However, this vermicompost also had the highest EC and the lowest pH along with more nitrogen in ammonia-N, and these characteristics, to a certain extent, may inhibit plant growth. Since the final effect of vermicompost on plant growth are the result of a balance results of vermicompost property, medium character, and plant reaction, changes in a small amount of amendments in initial material may not significantly alter the property of a vermicompost or their effects of vermicompost on plant growth.

Figure 4.16 Pak Choi plants grown in pots containing coir and vermicompost

* Four types of vermicomposts were mixed with coir at a rate of 20％, and the vermicomposts were treated with different processing methods.

Table 4.6 Growth parameters of Pak Choi grown in coir substituted with 20% of different vermicomposts with various processing methods (Part 1)

Treatment	Leaf area (cm^2)	Leaves	Stem	Root	Leaves	Stem	Root	LAR
		Fresh weight /g			Dry weight /g			
C	13.13 ± 4.01 h	0.40 ± 0.25 e	0.06 ± 0.02 g	0.03 ± 0.01 f	0.03 ± 0.01 e	0.01 ± 0.01 e	0.02 ± 0.01 d	467.01
Ff	223.33 ± 31.10 g	7.42 ± 0.86 d	1.57 ± 0.25 f	1.46 ± 0.50 e	0.84 ± 0.13 d	0.15 ± 0.02 d	0.22 ± 0.04 bc	284.31
Fs	256.67 ± 23.07 fg	9.31 ±1.01 bcd	1.86 ± 0.22 ef	1.41 ± 0.57 e	1.09 ± 0.30 bcd	0.18 ± 0.03 cd	0.23 ± 0.05 bc	247.09
Fcs	251.46 ± 48.03 fg	8.57 ± 1.77 cd	2.12 ± 0.58 cdef	1.97 ± 0.47 de	1.01 ± 0.28 cd	0.20 ± 0.05 bcd	0.22 ± 0.04 bc	256.23
Fscs	268.54 ± 61.64 efg	9.38 ± 1.86 bcd	1.97 ±0.38 def	2.55 ± 0.83 bcd	1.04 ± 0.25 cd	0.19 ± 0.04 cd	0.23 ± 0.09 bc	259.39
ODf	311.67 ± 45.99 bcdef	12.06 ± 2.77 ab	2.42 ± 0.55 bcde	1.54 ± 0.68 e	1.10 ± 0.23 abcd	0.20 ± 0.04 bcd	0.17 ± 0.05 c	288.04
ODs	391.04 ± 79.02 a	14.13 ± 4.53 a	3.29 ± 0.78 a	3.12 ± 0.76 ab	1.44 ± 0.39 ab	0.25 ± 0.05 ab	0.28 ± 0.05 ab	276.70
ODcs	346.88 ± 71.47 abcd	11.91 ± 3.40 ab	2.75 ± 0.88 abc	3.45 ± 0.31 a	1.19 ± 0.30 abcd	0.23 ± 0.07 abc	0.34 ± 0.05 a	294.69
ODscs	337.08 ± 76.35 abcde	11.66 ± 2.77 abc	2.59 ± 0.79 abcd	1.87 ± 1.00 de	1.12 ± 0.32 abcd	0.22 ± 0.08 bc	0.23 ± 0.12 bc	306.31
Sf	357.92 ± 108.23 abc	12.96 ± 3.37 a	2.76 ± 0.78 abc	1.47 ± 0.69 e	1.21 ± 0.40 abc	0.23 ± 0.05 abc	0.24 ± 0.07 bc	299.44
Ss	277.92 ± 93.10 defg	9.21 ± 3.72 bcd	2.01 ± 0.40 def	2.14 ± 1.00 cde	0.90 ± 0.43 cd	0.20 ± 0.07 bcd	0.22 ± 0.10 bc	354.96
Scs	298.54 ± 57.32 cdefg	9.51 ± 2.70 bcd	2.12 ± 0.84 cdef	2.55 ± 0.90 bcd	1.08 ± 0.25 bcd	0.18 ± 0.07 cd	0.26 ± 0.10 ab	278.43
Sscs	386.88 ± 84.19 ab	12.98 ± 12.98 a	3.10 ± 0.87 ab	2.88 ± 0.83 abc	1.46 ± 0.52 a	0.29 ± 0.08 a	0.33 ± 0.10 a	275.35

C : Controlled coir medium; Ff: Fresh vermicompost derived from fresh shredded wastes; Fs: Fresh vermicompost derived from fresh shredded wastes mixed with 20% sawdust; Fcs: Fresh vermicompost derived from fresh shredded wastes mixed with cow slurry; Fscs: Fresh vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding; ODf: Oven dried vermicompost derived from fresh shredded wastes; ODs: Oven dried vermicompost derived from fresh shredded wastes mixed with 20% sawdust; ODcs: Oven dried vermicompost derived from fresh shredded wastes mixed with cow slurry; ODscs: Oven dried vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding; Sf: Sterilised vermicompost derived from fresh shredded wastes; Ss: Sterilised vermicompost derived from fresh shredded wastes mixed with 20% sawdust; Scs: Sterilised vermicompost derived from fresh shredded wastes mixed with cow slurry; Sscs: Sterilised vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding.

Table 4.6 Growth parameters of Pak Choi grown in coir substituted with 20% of different vermicomposts with various processing methods (Part 2)

Treatment	Number of true leaves per plant	Root Length (cm)	R/S ratio	Chl. a	Chl. b	Chl. a / Chl. b	Total chl. content	Carotenoid
				mg/g				
C	3.00 ± 0.63 e	13.28 ± 1.52 d	0.55 ± 0.26 a	1.94 ± 0.03	0.33 ± 0.13	6.61	2.27 ± 0.12	0.80 ± 0.03
Ff	7.00 ± 0.63 cd	17.46 ± 1.97 abc	0.22 ± 0.05 b	7.18 ± 0.29	1.89 ± 0.32	3.89	9.08 ± 0.21	2.93 ± 0.07
Fs	6.83 ± 0.75 d	16.31 ± 3.43 bcd	0.18 ± 0.03 b	3.83 ± 0.43	1.53 ± 0.37	2.64	5.37 ± 0.23	1.67 ± 0.13
Fcs	7.33 ± 1.21 bcd	18.67 ± 1.92 abc	0.19 ± 0.02 b	6.26 ± 0.05	1.73 ± 0.25	3.68	8.00 ± 0.23	2.47 ± 0.08
Fscs	7.67 ± 0.82 abcd	16.26 ± 1.83 bcd	0.19 ± 0.08 b	6.72 ± 0.10	1.68 ± 0.16	4.03	8.42 ± 0.21	2.73 ± 0.08
ODf	7.67 ± 0.82 abcd	19.54 ± 2.34 a	0.13 ± 0.03 b	7.68 ± 0.22	2.05 ± 0.34	3.82	9.75 ± 0.34	3.02 ± 0.12
ODs	8.67 ± 1.51 a	15.57 ± 1.19 cd	0.17 ± 0.03 b	7.48 ± 0.35	2.05 ± 0.36	3.73	9.55 ± 0.43	3.00 ± 0.12
ODcs	8.00 ± 0.89 abc	16.99 ± 1.76 abc	0.24 ± 0.05 b	6.54 ± 0.31	1.90 ± 0.38	3.57	8.46 ± 0.20	2.65 ± 0.09
ODscs	7.50 ± 0.55 bcd	15.82 ±3.03 bcd	0.17 ± 0.05 b	7.80 ± 0.22	2.04 ± 0.32	3.90	9.86 ± 0.43	2.98 ± 0.17
Sf	8.17 ± 0.75 ab	19.48 ± 4.19 a	0.17 ± 0.03 b	7.80 ± 0.31	1.91 ± 0.40	4.23	9.73 ± 0.41	2.93 ± 0.13
Ss	7.00 ± 1.26 cd	18.87 ± 3.83 ab	0.17 ± 0.04 b	7.71 ± 0.11	1.93 ± 0.35	4.10	9.65 ± 0.27	2.72 ± 0.05
Scs	8.33 ± 1.03 ab	17.32 ± 3.29 abc	0.21 ± 0.09 b	7.58 ± 0.31	2.03 ± 0.59	4.02	9.63 ± 0.74	2.84 ± 0.13
Sscs	8.00 ± 0.89 abc	17.91 ± 2.72 abc	0.20 ± 0.08 b	8.25 ± 0.30	2.01 ± 0.67	4.52	10.28 ± 0.74	3.21 ± 0.08

* All values are in Means ± Std Deviations (n=6).

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student’s t-test), and indicated by different letters.

Although there were no significant difference between the four types of vermicompost on plant growth, the distribution of Pak Choi dry weight seemed to correlated with the processing method (Figure 4.17). Generally, plants treated with sterilised vermicompost (S) were prone to have a lower leaf dry weight percentage, with half of the S treatments lower than 70%, while treatments of F and OD are all over 70%. In addition, it is interesting to note that plants with sterilised vermicompost addition also had a higher Chl.a/ Chl.b ratio. Sumanta et al.(2014) demonstrated that chlorophyll a/b can be a sensitive biomarker of pollution and environmental stress. Therefore, this result suggests that S treatments produced a higher environmental stress than F and OD treatment. Also, Am-Euras (2009) documented a loss of 'stimulatory effect' when vermicompost was sterilised. He also pointed out the role of growth promoting plant hormones in vermicompost such as gibberellins, auxins, and cytokinins, which were secreted from both earthworms and microbes. Thus, it can be speculated that the changes in chlorophyll content and dry weight distribution for the S treatments were likely due to the inactivation of these growth hormones and the death of microorganisms during the sterilising process.

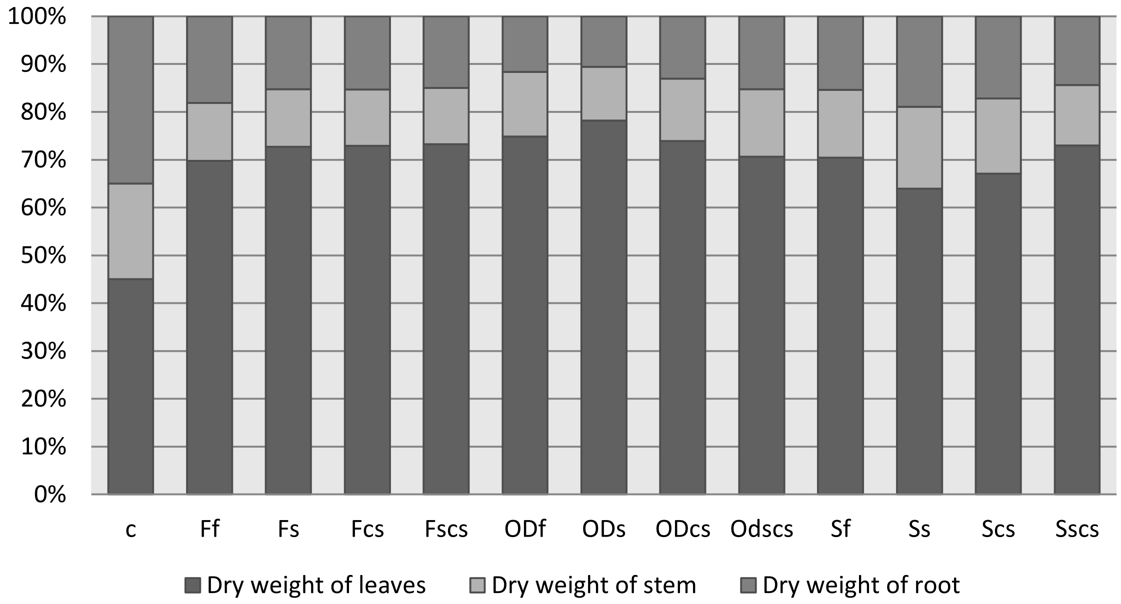

Figure 4.17 Distribution of Pak Choi dry weight in different treatments

C : Control; Ff: Fresh vermicompost derived from fresh shredded wastes; Fs: Fresh vermicompost derived from fresh shredded wastes mixed with 20% sawdust; Fcs: Fresh vermicompost derived from fresh shredded wastes mixed with cow slurry; Fscs: Fresh vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding; ODf: Oven dried vermicompost derived from fresh shredded wastes; ODs: Oven dried vermicompost derived from fresh shredded wastes mixed with 20% sawdust; ODcs: Oven dried vermicompost derived from fresh shredded wastes mixed with cow slurry; ODscs: Oven dried vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding; Sf: Sterilised vermicompost derived from fresh shredded wastes; Ss: Sterilised vermicompost derived from fresh shredded wastes mixed with 20% sawdust;

Scs: Sterilised vermicompost derived from fresh shredded wastes mixed with cow slurry; Sscs: Sterilised vermicompost derived from fresh shredded wastes mixed with 20% used animal bedding.

4.8 Pot Experiment 6: The influence of added chemical fertiliser to vermicompost on the performance of Pak Choi growth

4.8.1 Treatments and experimental design

The final experiment was to investigate how vermicompost interacts with chemical fertiliser. The experiment lasted for 20 days from 26 March to 15 April. During the experimental period, the temperature in the greenhouse ranged from 13°C to 30°C with a mean temperature of 20.8°C. The vermicompost used in the experiment was FS + 20% UAB (for details see 4.2.2), and a coir-based medium used as the main potting substrate. There were three levels of chemical fertiliser addition, half (1.5g/L), full (3g/L), and double (6g/L) of recommended rate for general plant growth (Osmocote Extract, 3-4 month release, 16-3.5-10). Each of these levels was either tested alone or mixed 20% vermicompost. A control without chemical fertiliser amendment was also used. Plants were grown in 200ml plastic pots as per Pot Experiment 5, and there were six replicates for each treatment.

4.8.2 Experimental procedure and assessments

Different amounts of chemical fertiliser (Osmocote Extract, 3-4 month release, 16-3.5-10) were weighted according to required level of addition and the size of the container. In total, there were eight treatments: pure coir (C; control1), coir mixed with 20% vermicompost (vc, control 2), coir mixed with half recommended chemical fertiliser (Hcf; 1.5g/L), coir mixed with 20% vermicompost and half recommened chemical fertiliser (Hcf + vc; 1.5g/L), coir mixed with full chemical fertiliser that recommened (Fcf; 3g/L), coir mixed with 20% vermicompost and full chemical fertiliser that recommened (Fcf + vc; 3g/L), coir mixed with double rate of recommended chemical fertiliser (Dcf; 6g/L), coir mixed with 20% vermicompost and double rate of recommended chemical fertiliser (Dcf + vc; 6g/L). The weighted chemical fertiliser was mixed with coir and vermicompost following the treatments above. The pots was then filled with the prepared mixture, and uniform Pak Choi seedlings were selected and transplanted to the pots. The seedlings had been raised in a standard seed-starting mix for about ten days as described in Pot Experiment 5. The established plants were watered daily at a rate of 30 ml. Plants were harvested on the 20th day after transplantation and measured for various growth parameters including chlorophyll content, leaf area, shoot length, root length and fresh/ dry weight.

4.8.3 Results and discussion

Figure 4.18 shows Pak Choi plants grown in coir amended with vermicompost and chemical fertiliser (Osmocote Extract, 3-4 month release, 16-3.5-10) in different ranges.

Generally, plants amended with both vermicompost and chemical fertiliser (Dcf + vc, Fcf + vc, Hcf + vc) had the greatest improvement in plant growth (dry weight, leaf number and leaf area) followed by pure vermicompost (vc), chemical fertilisers (Dcf, Fcf, Hcf) and control (c) (Table 4.7). A significant lower number of leaves, leaf area and dry weight was observed for plants treated with chemical fertiliser alone compared to vermicompost treatments. However, the same amount of chemical fertiliser (3 kg/m^3) amended in bark /pumice-based medium (standard potting mix) produced much better plants (Pot Experiment 3). This demonstrated that the chemical fertiliser use efficiency in the coir-based medium was quite low, and most of the chemical fertiliser could not be take up by the plants. Similar results were reported for common purslane when it was grown in coir amended with synthetic fertiliser (liquid fertiliser) (Cros et al., 2007). In their experiment, the plant height and fresh weight of common purslane grown in coir were significantly lower than plants grown in peat and other media. It was noticeable that a slight reduction in leaf number was observed when the chemical fertiliser application rate was raised from 1.5 kg/m^3 (Hcf) to 3 kg/m^3 (Fcf). However, significant increases in leaf number and area were also observed when the application rate was at 6 kg/m^3 (Dcf).

Vermicompost application resulted in significant increases in plant growth. All treatments with vermicompost addition (vc, Dcf + vc, Fcf + vc, Hcf + vc) had a significantly higher leaf number, leaf area and plant dry weight than the pure chemical fertiliser treatments (Dcf, Fcf, Hcf) (Table 4.7). In addition, mixing chemical fertiliser and vermicompost together led to a further improvement in plant growth in comparison with the use of vermicompost alone. However, it was noticeable that the increase in plant dry weight ceased when the amount of chemical fertiliser amended with vermicompost was at 6 kg/m^3 compared to 3 kg/m^3, although the leaf number and leaf area did increase. This result indicates that plants may uptake nutrients more easily from vermicompost, and it could be a good strategy to use vermicompost and chemical fertiliser in horticultural production.

Figure 4.18 Pak Choi responses to different ranges of vermicompost and chemical fertiliser additions

* Dcf+vc: Coir-based medium containing 6Kg/m^3 Osmocote® Extract (16-3.5-10) with 20% vermicompost (drerived FS+20%UAB); Fcf+vc: Coir-based medium containing 3Kg/m^3 Osmocote® Extract with 20% vermicompost; Hcf+vc: Coir-based medium containing 1.5Kg/m^3 Osmocote® Extract with 20% vermicompost; vc: coir mixed with 20% vermicompost; C: Coir medium as a control; Dcf: Coir amended with 6Kg/m^3 Osmocote® Extract; Fcf: Coir amended with 3Kg/m^3 Osmocote® Extract; Hcf: Coir amended with 1.5Kg/m^3 Osmocote® Extract.

Table 4.7 Growth parameters of Pak Choi plants with different ranges of vermicompost and chemical fertiliser additions

Treatment	Number of true leaves per plant	Leaf area (cm^2)	R/S Ratio	LAR	Average dry weight of plant
C	3.00 ± 0.63 e	13.13 ± 4.01 f	0.55 ± 0.26 a	467.01	0.06 ± 0.03 d
vc	6.67 ± 1.03 b	251.67 ± 53.52 c	0.21 ± 0.09 bc	267.38	1.49 ± 0.43 b
Hcf	4.17 ± 0.75 d	62.71 ± 22.27 e	0.21 ± 0.07 bc	273.22	0.48 ± 0.43 c
Fcf	3.83 ± 1.47 d	84.79 ± 57.47 e	0.28 ± 0.06 b	254.12	0.49 ± 0.48 c
Dcf	5.50 ± 0.55 c	140.83 ± 32.40 d	0.25 ± 0.11 bc	278.06	0.81 ± 0.21 c
Hcf + vc	8.17 ± 0.75 a	322.50 ± 44.34 b	0.16 ± 0.04 c	268.63	1.72 ± 0.27 ab
Fcf + vc	7.50 ± 0.55 ab	335.21 ± 44.28 ab	0.22 ± 0.06 bc	246.32	2.00 ± 0.38 a
Dcf + vc	8.17 ± 1.33 a	376.46 ± 29.19 a	0.22 ± 0.09 bc	282.26	2.00 ± 0.38 a

* All values are in Means ± Std Deviations (n=6).

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t test), and indicated by different letters.

* C: Coir medium as a control; vc: coir mixed with 20% vermicompost (derived FS+20%UAB); Hcf: Coir amended with 1.5Kg/m^3 Osmocote® Extract (16-3.5-10); Fcf: Coir amended with 3Kg/m^3 Osmocote® Extract; Dcf: Coir amended with 6Kg/m^3 Osmocote® Extract; Hcf+vc: Coir-based medium containing 1.5Kg/m^3 Osmocote® Extract with 20% vermicompost; Fcf+vc: Coir-based medium containing 3Kg/m^3 Osmocote® Extract with 20% vermicompost; Dcf+vc: Coir-based medium containing 6Kg/m^3 Osmocote® Extract with 20% vermicompost.

Table 4.8 shows the chlorophyll and carotenoid contents of Pak Choi leaves. Since chlorophyll content and chlorophyll a/b ratio are considered as a sensitive biomarker of pollution and environmental stress (Sumanta et al., 2014), chlorophyll content could reflex the effects of fertiliser addition on plant growth and development. Pak Choi plants grown in coir with only chemical fertiliser additions (Hcf, Fcf, Dcf) generally had a lower chlorophyll and carotenoid content, with a higher chlorophyll a/b ratio compared to vermicompost treatments (vc, vc+Hcf, vc+Fcf, vc+Dcf). Chemical fertiliser additions resulted in further improvements on chlorophyll (a and b) and carotenoid content for treatments amended with both chemical fertiliser and vermicompost (vc+Hcf, vc+Fcf, vc+Dcf) compared to vermicompost only (vc).

It was noticeable that there was a reduction in chlorophyll a content when the application rate of chemical was raised from 3Kg/m^3 (F) to 6Kg/m^3 (D) (Figure 4.19). This result suggests that there may be negative effects on chlorophyll content which will have further effects on plant photosynthesis when excess chemical fertilisers is added to the coir-based medium.

Overall, these results revealed that there might be a complex interaction between vermicompost and chemical fertiliser addition on plant growth, and the ideal fertiliser combination for a coir-based medium was vc+Fcf, considering fertiliser use efficiency and the effects on chlorophyll content.

Table 4.8 Chlorophyll and carotenoid content of Pak Choi plants

Treatment	Chl. a	Chl. b	Chl. a / Chl. b	Total chl.	Carotenoid
C	1.94 ± 0.03	0.33 ± 0.13	6.61	2.27 ± 0.12	0.80 ± 0.03
vc	5.67 ± 0.32	1.63 ± 0.14	3.51	7.32 ± 0.19	2.19 ± 0.21
Hcf	3.93 ± 0.21	1.19 ± 0.07	3.31	5.13 ± 0.25	1.48 ±0.30
Fcf	6.07 ± 0.19	1.81 ± 0.47	3.57	7.90 ± 0.34	2.31 ± 0.50
Dcf	5.51 ± 0.24	2.37 ± 0.08	2.33	7.89 ± 0.20	2.51 ± 0.04
Hcf + vc	6.73 ± 0.47	2.62 ± 0.56	2.64	9.37 ± 0.71	2.71 ± 0.56
Fcf + vc	6.82 ± 0.41	2.42 ± 0.43	2.89	9.26 ± 0.15	2.76 ± 0.30
Dcf + vc	6.28 ± 0.51	2.36 ± 0.54	2.80	8.65 ±0.10	2.67 ± 0.43

* All values are in Means ± Std Deviations (n=6).

* Statistical analysis of different groups was carried out with significance (least significant difference) at 0.05, and significant differences determined by ANOVA and post-hoc multiple comparison tests (Student's t-test), and indicated by different letters.

* C: Coir medium as a control; vc: coir mixed with 20% vermicompost (derived FS+20%UAB); Hcf: Coir amended with 1.5Kg/m^3 Osmocote® Extract (16-3.5-10); Fcf: Coir amended with 3Kg/m^3 Osmocote® Extract; Dcf: Coir amended with 6Kg/m^3 Osmocote® Extract; Hcf+vc: Coir-based medium containing 1.5Kg/m^3 Osmocote® Extract with 20% vermicompost; Fcf+vc: Coir-based medium containing 3Kg/m^3 Osmocote® Extract with 20% vermicompost; Dcf+vc: Coir-based medium containing 6Kg/m^3 Osmocote® Extract with 20% vermicompost.

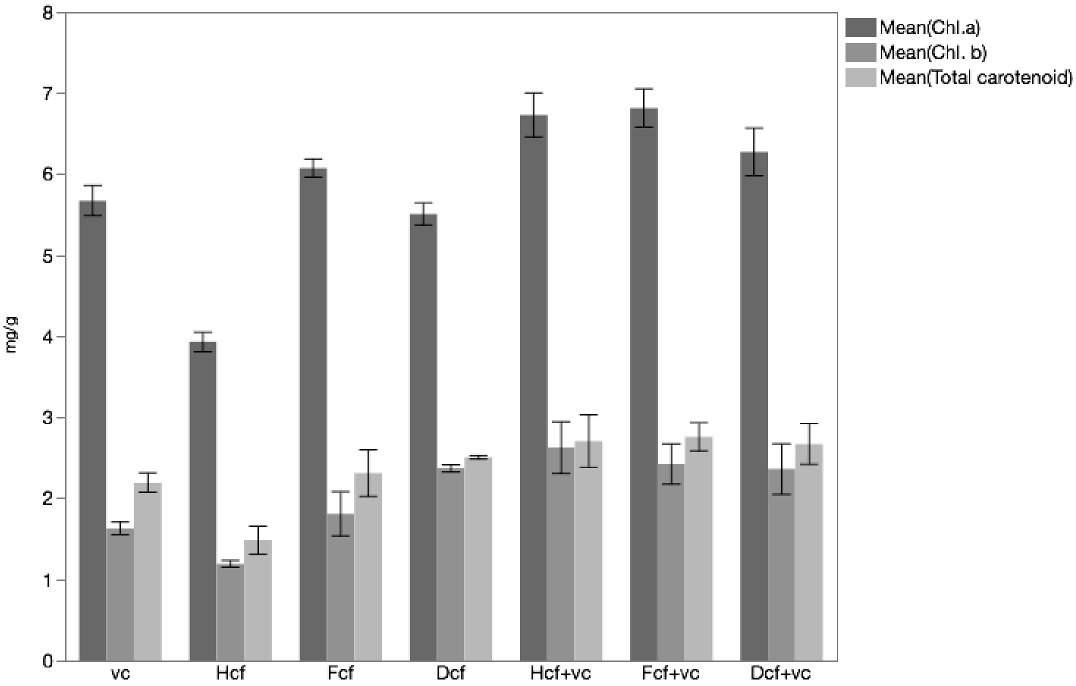

Figure 4.19 Content of chlorophyll a, chlorophyll b and carotenoid in all treatments

* Each error bar is constructed using standard error from the mean.

* vc: coir mixed with 20% vermicompost (drerived FS+20%UAB); Hcf: Coir amended with 1.5Kg/m^3 Osmocote® Extract (16-3.5-10); Fcf: Coir amended with 3Kg/m^3 Osmocote® Extract; Dcf: Coir amended with 6Kg/m^3 Osmocote® Extract; Hcf+vc: Coir-based medium containing 1.5Kg/m^3 Osmocote® Extract with 20% vermicompost; Fcf+vc: Coir-based medium containing 3Kg/m^3 Osmocote® Extract with 20% vermicompost; Dcf+vc: Coir-based medium containing 6Kg/m^3 Osmocote® Extract with 20% vermicompost.

Chapter 5: Summary and Overall Discussion

5.1 Summary

The research was a fundamental study to investigate the feasibility of vermicomposting domestic waste and the effects of the waste generated vermicomposts on plant growth to consider the value of vermicomposting technology in waste management and horticultural production in the future.

Two vermicomposting experiments and six pot experiments were conducted over the course of a year. The experiments focused on testing the influences of the composition of different wastes on earthworm growth and on how other possible factors could affect the performance of vermicompost applications on plant growth.

Based on two problematic waste streams in the Canterbury region, a mixture of municipal organic waste and used animal bedding was considered as an available food source for raising earthworms. Fresh shredded waste (FS) was the most suitable material as a component of earthworm food compared with hot rotted waste (HR) and old shredded waste (OS). Earthworms raised in fresh shredded waste had a significantly higher reproductive rate (a greater cocoon production and a higher number of juvenile earthworms). Another waste, used animal bedding (UAB), showed consistently positive influences on earthworm reproduction and survival rates when mixed with the three types of municipal waste up to a proportion of 20%, although earthworms could not survive in the UAB waste alone (used animal bedding).

The composition of feeding materials also influenced the nutrient content of final vermicomposts. Vermicompost derived from FS had the highest level of nitrogen content. HR-generated vermicomposts had a significantly lower level of ammonia and nitrate content with only half amount of nitrogen content than those from FS. In addition, there was a complex interaction between types of municipal waste and the amount of used animal bedding that had been mixed in. When the amount of used animal bedding was increased (up to 20%), ammonia-N was transformed to Nitrate-N in HR-generated vermicompost, but an opposite effect was recorded for vermicomposts derived from FS. A sharp drop in total nitrogen content (in both ammonia-N and Nitrate-N) was observed when 80% OS was mixed with 20% UAB. These results indicated that the nitrogen content of vermicompost could be influenced by the composition of the raw materials by altering the nitrogen cycle.

The composition of feeding materials not only affects the growth and reproduction of adult earthworms, but it also influences the development of their following generation. This study found that FS was the best type of waste for the growth and development of the second earthworm generation in terms of cocoon viability and the number of earthworms produced per cocoon. The effects of UAB substitution were variable in FS-based and HT-based vermicomposting systems in terms of cocoon viability, the number of earthworms per cocoon and the number of newly generated earthworms. This result implies that there was complex interaction between the amount of UAB substitution and the type of municipal wastes. It was interesting that a 50% UAB substitution resulted in a major reduction in the cocoon viability, the number of earthworms per cocoon and the number of newly generated earthworms for both FS and HT treatment. Also, a 40% UAB substitution appeared to be the best rate for the growth and development of new earthworm generation. However, there was no much difference between the 40% UAB substitution and 20% UAB substitution for FS-based treatments in term of cocoon viability and the number of regenerated earthworms. Thus, for practical purpose, 20% used animal bedding mixed with 80% fresh shredded waste was an ideal combination to use on recycling the wastes.

In this study, pot trials were also set up to examine the effects of these waste-derived vermicomposts on plant growth with testing various influencing factors.

Generally, there was significant growth promoting effects on plants grown in substrates with added vermicompost. Vermicompost mixed with vermiculite and coir had a higher use efficiency than sand and peat. This indicated complex interactions between the physico-chemical characteristics of the potting medium and vermicompost addition.

Vermicompost derived from domestic organic waste had a significantly lower plant available nitrogen content (about 20 times less) than vermicompost derived from fruit and vegetable waste. Due to the low nitrogen content, plants grown in the substrate with 5% waste-derived vermicomposts addition had a lower plant dry weight than those amended with vermicompost derived from fruit and vegetable waste. However, vermicompost type had less influence on plant growth when artificial nutrients were added. For plants grown in the standard potting mix amended with vermicompost derived from old shredded waste (OS), there was a significant reduction in shoot length. The reason for the reduction in shoot length is unknown but may have been due to the content of hazardous elements, changes in the physical structure of the potting medium, or an excess content of growth regulators. Further research is needed to confirm the reason for the effects.

The concentration of vermicompost can significantly affect plant growth. An increased vermicompost application up to 30% resulted in a greater vegetative growth of Pak Choi plants. Additionally, an interaction between the medium types and concentration of vermicompost was also noticed. Coir (C-) appeared to have higher vermicompost use efficiency than the bark/pumice-based medium (P-: standard potting mix minus fertilisers).

The method of vermicompost application also influenced on vermicompost use efficiency. These effects depend on the type of medium used. The most efficient application method for coir-based medium (C-) was by mixing vermicompost into the medium (M), while surface application (S) appeared the best for the bark/pumice-based medium (P-) with significantly higher average leaf number (3.67) and plant dry weight (62.6mg) compared to the mixed treatment (2.67, 39.6mg).

For the processing methods, three methods (fresh harvested, oven dried and sterilised) were tested. Freshly harvested vermicomposts had less promoting effects on Pak Choi growth with lower leaf area and plant dry weight. This may be due to nutrient releases from microorganisms in the drying and sterilising processes. In addition, plants grown in coir amended with sterilised vermicomposts have a slightly lower leaf dry weight percentage with a higher chlorophyll a/b ratio. These changes may have been due to the inactivation of plant growth hormones and the death of microorganisms during the sterilising process.

The final pot experiment tested the compatibility of vermicompost with chemical fertiliser. Coir showed a particularly low artificial fertiliser use efficiency, and Pak Choi did not grow well even when the chemical fertiliser supply has been double than the recommended rate. However, plants seemed to uptake nutrients more easily from vermicompost when it was mixed with coir, and the utilisation rate of the chemical fertiliser was also increased when these combined with vermicompost.

5.2 Overall discussion

Although short discussions have been made separately for each experiment, some findings across several experiments have not been covered. These will be discussed in this chapter.

5.2.1 Influences of raw materials on earthworm morphology and biotic community in vermicomposting systems

In addition to the effects of the raw materials on earthworm growth and reproductive rate, influences on earthworm morphology and on the biotic community were also observed in all vermicomposting experiments.

The composition of the raw materials used was found to affect the biotic community in the vermicomposting system, and changes in these communities were observed in all the vermicomposting experiments. These observations indicated complex interactions among living organisms in the vermicomposting systems. For example, sporocarps, known as fruit body of fungi, were found in the HT-based (hot rotted waste) and FS-based (fresh shredded waste) vermicomposting systems, but were rarely observed in the OS-based (old shredded waste) system (Figure 5.1: A). Weeds and insects were also observed during the vermicomposting process, and may cause concern about vermicompost quality especially if these wastes are used as raw materials in commercial vermicompost production.

It was also found that the morphology of earthworms was affected by raw material used for vermicomposting. Earthworms feed on old shredded waste had a darker colour, and also seemed to be more sensitive to disturbances than earthworms in the fresh shredded waste and the hot rotted waste. Earthworms in old shredded waste had a "swollen clitellum" and produced a smaller cocoon size (Figure 5.1: B, C). These negative effects on the sexual development of earthworms may have lead to the low reproductive rate of earthworms in old shredded waste. Although it is not known what cause the negative effects of old shredded waste, several studies have mentioned that certain substances can have negative effects on earthworms. For example, Sinha et al. (2010) reported that earthworms are sensitive to the salt content of feeding materials; Gunadi and Edwards (2003) demonstrated feed materials with high electrical conductivity, high NH_4 content, high moisture content or low pH can be fatal for *Eisenia foetida*. Yadav & Garg (2010) pointed out that high levels of ammonia, salt, polyphenols, inert materials, heavy metals, glass, plastics, pharmaceuticals and detergents are toxic to earthworms. Thus, the negative effects of the old shredded waste may be explained by these unfavourable factors or substances in it.

Despite the gaps in knowledge still exist, the findings presented here on the changes in earthworm morphology and biotic community may provide a view to future studies, especially for evaluating the toxicity of some compounds to earthworms. This could further our understanding of the mechanisms of vermicomposting.

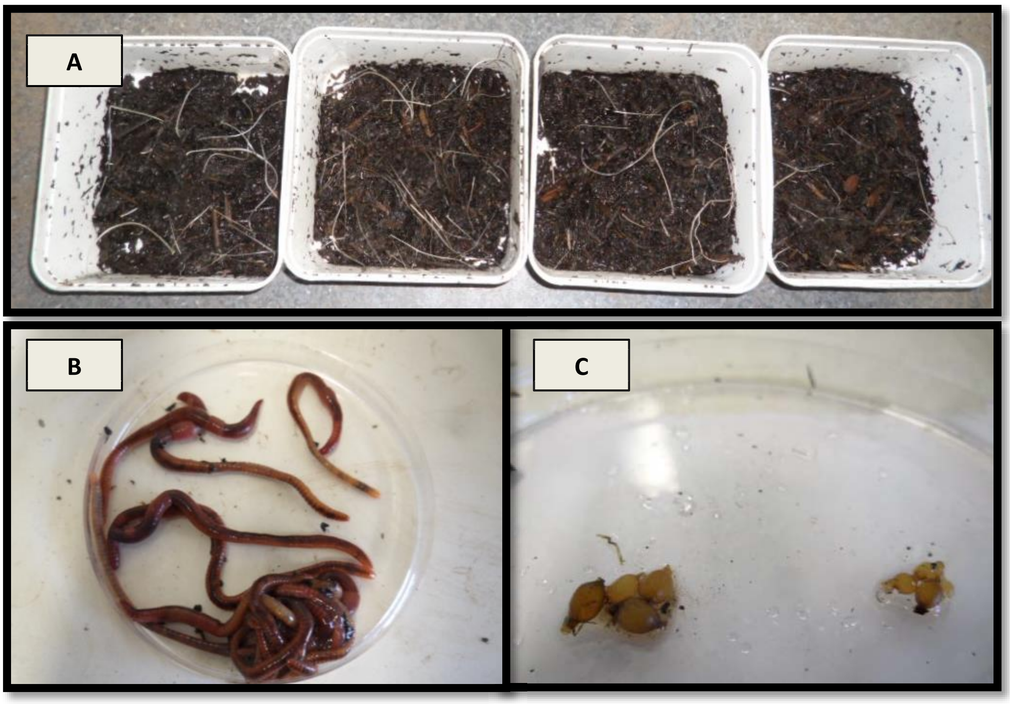

Figure 5.1 Influences of raw material on vermicompost biotic community and earthworm morphology

* A: Growth of sporocarps in HR-based (hot rotted waste) vermicomposting system; B: Earthworms with swollen clitellum from OS-based (old shredded waste) vermicomposting system; C: Differences in cocoon size from different vermicomposting systems (left: cocoons from FS-based (fresh shredded waste) vermicomposting system, right: cocoons from OS-based vermicomposting system).

5.2.2 Interactions between the characteristics of vermicompost raw materials and earthworms

As mentioned earlier, the raw materials used in the experiments of this study were three types of municipal waste (fresh shredded waste, hot rotted waste and old shredded waste) and used animal bedding from a dairy industry. Although the waste types were tested in the experiments, the effects of these waste types on earthworm growth seem to be more related to the characteristics of the raw materials rather than the types of waste. The effects of raw material (material types) to earthworm growth seem to have been influenced by the pre-composting time, as the characteristics of raw materials change with vermicomposting time.

It was found that the different types of municipal waste had distinctive effects on earthworm growth and development. However, the three wastes used were from the same source but were treated with different composting methods and time. Fresh shredded waste (FS) were found to be the most suitable material for earthworm growth and reproduction, followed by hot rotted waste (HR) and old shredded waste (OS). In other words, the material became less suitable for earthworms with increased vermicomposting time. This result is in agreement with the study of Frederickson et al. (1997) who found reductions in biomass and reproductive rate of the earthworm, *Eisenia andrei*, and with the increased pre-composting time for green wastes. The green wastes without pre-composting showed the best on earthworm growth and reproduction. Nevertheless, the authors proposed that vermicomposting alone, without a thermophilic stage, may not be enough to control pathogens, and suggested pre-composing should be kept to a minimum time but with effective sanitisation of the waste used. Such sanitisation problems were also seen in this study (weeds and insects), but it is not know how they will affect the use of vermicompost in horticultural production. Further studies are needed to achieve effective sanitisation without generating too many negative effects on earthworms and to test how the sanitisation of vermicompost can affect plant growth.

The vermicomposting process basically is an earthworm involved composting process, where the raw materials (initial wastes) are gradually stabilised. Previous studies have recorded changes in earthworm growth and reproduction during vermicomposting. For example, Frederickson et al. (1997) reported a reduction in earthworm weight after 7 weeks of vermicomposting (4 earthworms in 200g of green waste). Also, decreases in average earthworm biomass after 4 weeks were described by Fernandez-Gomez et al. (2010) using damaged tomato mixed with cow dung or straw (in different proportions 2:1 and 4:1) as feeding materials (5 non-clitellated earthworms in 0.5L of each wastes). Garg & Gupta (2011) found decreases in mean individual biomass after vermicomposting for 8 or 9 weeks when earthworms were fed on cow dung and pre-consumer processing vegetable waste (20 adult earthworms in 1kg of feed mixture). In addition, in this study, a reduction in earthworm biomass using municipal wastes mixed with used animal bedding in different proportions after vermicomposting for 20 days (13 adult earthworms in 200g feeding substrates) was also observed.

From these findings, it can be seen that the growth of earthworms is correlated with the nutrient status of raw materials used, as has been proposed in prior studies (Manna et al., 1997; Yadav and Garg, 2011). Also, it was noticeable that not only nutrient content (source of raw material) is crucial for the growth of earthworms, but the amount of raw materials and number of earthworms (earthworm density) are also important. Furthermore, Gunadi and Edwards (2003) observed that

Eisenia foetida tended to decrease in weight after 60 weeks of vermicomposting, although continuous feeding materials (with new substrates added) were adding to the vermicomposting system. This indicated that not only can the nutrient status of raw materials limit the growth of earthworms, but that some substances produced by the degradation of raw materials may also be harmful to earthworm growth. However, currently little scientific work has been carried out on the dynamics of toxic substance accumulation in the vermicomposting process, and further studies are needed in this area.

5.2.3 Influence of vermicompost and growing medium on plant growth

Six pot trials were set up to test factors influencing the effects of vermicompost on plant growth. These potting experiments were conducted to examine possible factors that may influence the performance of vermicompost on plant growth including medium type, source, concentration, application method, processing method and compatibility with chemical fertiliser, respectively. Although the results were mostly consistent with previous research, some new findings may further our understanding of the interaction between vermicompost and plant growth.

Generally, there were positive effects of vermicompost application in all pot experiments, which agree with the findings of previous research, that vermicompost application results in improvements in the growth and development of plants (Joshi et al., 2015). Nevertheless, many previous studies only focused on the influence of the source and concentration of the vermicompost on plant growth, while little work has been done on the effects of the other influencing factors. For example, an application of 5% vermicompost derived from pig manure and a mixture of pig and cattle manure (50:50) in a commercial plant growth medium (Metro-Mix 360) significantly promoted the growth of tomato plants (Atiyeth et al., 1998; Atiyeth et al., 2000). It was proposed that this effect was not only attributed to the nutritional and physical properties of the vermicompost used but was also linked with other biological changes, such as an increase in plant growth regulator content of the growth medium. In this study, a significant increase (38%) in seedling dry weight was found when Pak Choi seedlings were grown in a standard potting mix (with full nutrients supplied) substituted with 5% vermicompost (Pot Experiment 2). This finding is in agreement with the previous research that vermicompost can further promote plant growth even when adequate chemical nutrients have been provided.

It was also found in this study that, although the 5% vermicompost supplement in the standard potting mix resulted in a significant improvement in Pak Choi growth, the type of vermicompost (nutrient content) did not have any effect on plant growth. This result provided extra evidence to support the hypothesis that the physical and biological properties of the growing medium are crucial to the growth promoting effects of vermicompost.

However, when Pak Choi seedling was grown in a coir medium without the addition of chemical fertiliser, plant growth was found to be more related to the source and the nutrient content of the vermicompost. This finding indicated that the nutrient content of vermicompost can also play an important role in its effects on plant growth, especially when plants have not had a sufficient nutrient supply.

The most significant improvement in Pak Choi growth occurred when the vermicompost application rate was increased from 5% to 10%. The plant growth responses to the vermicompost addition appeared similar to those with chemical fertiliser, following the law of diminished returns, especially when vermicompost was the only source of nutrient supply (Pot Experiment 3). These results are consistent with previous findings that the growth of plants does not always increase with the amount of vermicompost added (Edwards et al., 2011). However, a limitation of the study on vermicompost concentration was that the range of concentrations tested was relatively small (0-30%). This range was chosen because it is not economically viable to use high vermicompost application rate in horticulture production. Nevertheless, it is likely that negative effects may occur with a high level of vermicompost concentration, since in this study the growth promoting effects of vermicompost almost ceased when the application rate was about 30%, especially with the coir-based medium.

The effects of vermicompost on Pak Choi growth was found to be quite variable between the different media tested. These media also showed different responses to the changes in vermicompost application rate. Coir appeared to have a higher vermicompost use efficiency than a bark/pumice-based medium when vermicomposts were mixed in. In Pot Experiment 4, surface application appeared to be the best for the bark/pumice-based medium in terms of plant dry weight (62.6mg), compared to the mixed treatment (39.6 mg). Thus, Pak Choi plants grown in a bark/pumice-based medium may have higher vermicompost use efficiency if vermicompost was applied to the surface of the medium rather than mixed into the medium in the Pot Experiment 3. These findings suggest that there are interactions between medium type, vermicompost application rate and application method.

Pot Experiment 1 tested the plant performance of vermicompost application using different media. Pak Choi grown in vermiculite and coir had a higher vermicompost/nutrient use efficiency than in sand and peat moss. This result differs from previous studies which found that vermicompost and peat moss have complementary properties (Edwards et al., 2011). This contradictory result may be due to the differences in the composition of peat moss, the source of vermicompost and the species of the plant used in the experiments.

Pot Experiment 6 tested the influence of chemical fertiliser supplements in a coir-based growing medium. Currently, no published study tested the effects on plant growth of coir mixed with vermicompost on plant growth. This is probably because the use of coir in horticulture is still considered as a new technology, and some problems have been observed when plants are grown in a coir medium. For instance, Cros et al. (2007) documented that common purslane (*Portulaca oleracea*) grew poorly on coir compared with peat moss. Arenas et al. (2002) reported a reduction in plant growth when the amount of coir was over 50% in the substrate. Holman et al. (2013) reported that plants grown in coir substrate suffered from chlorosis, and proposed these negative effects were likely due to the high EC (salt content) of the coir. These findings agree with the observations here that Pak Choi did not grow well on coir, even when the chemical fertiliser supply was double the recommended rate (Pot Experiment 6). Vavrina et al. (1996) reported that coir has a low cation exchange capacity, and the extra nitrogen fertiliser may be needed for coir-grown plants to compensate for nitrogen immobilisation in the medium. Thus, the low chemical fertiliser use efficiency in the coir medium may be due to the high nitrogen immobilisation rate.

It is interesting that the utilisation rate of chemical fertiliser was increased when it combined with vermicompost (Pot Experiment 6). It seems that the vermicompost application in coir medium may increase the nutrient accessibility of plants, and the nutrients in vermicompost are less immobilised in coir medium. This may be due to the changes in chemical, physical and biological properties of coir after vermicompost application. However, Pot Experiment 4 showed that there was no significant difference between the two application methods S (surface application) and M (mixing with medium) for coir, in terms of plant dry weight, despite the M treatment producing a significantly higher leaf number and shoot length. This result indicated that the changes in physical property by vermicompost application may make minor influences on plant dry weight since the physical properties of coir will not be changed when vermicompost is applied on the surface. On the other hand, in Pot Experiment 5, the effects of sterilised vermicompost on Pak Choi growth were not different to oven dried vermicompost and even higher than fresh harvested vermicompost. This finding suggested that the changes in biological properties (growth hormone content and microbial activities) of coir-based media may not directly result in an increase in plant dry weight. Therefore, it is possible to hypothesise that complex interactions may exist among the chemical, physical and biological changes in the medium after vermicompost application, and the final growth promoting effects of vermicompost may be due to the interactions of these changes. However, the mechanisms of these interactions are still not know, and further works are needed to fill these knowledge gaps.

In summary, vermicompost derived from domestic wastes can be a promising fertiliser source for use in horticultural production. Coir appears to be a promising material to mix with vermicompost for use in crop production, and this result was not demonstrated in previous studies. Nevertheless, there are still some limitations in this study. Firstly, the size of the pot experiments and replicates were quite small, and a larger study may need to be conducted to confirm some of the hypotheses generated in this study. Secondly, the experiments only focused on certain influencing factors that may affect the performance of vermicompost in practical use. The mechanisms behind the factors, however, were not covered in this study. To understand these mechanisms, long-term and systematic experiments are required in the future.

Chapter 6: Conclusions and Recommendations for Future Research

6.1 Conclusion

- This study showed that it is feasible to recycle two waste streams together by combining municipal wastes and a dairy industry waste (used animal bedding) as a feeding substrate for use in vermicomposting, and positive cross effects were found when the two wastes were mixed together. Fresh shredded waste was the most suitable type to use in vermicomposting in preference to hot rotted waste or old shredded waste, with the ideal combination being 80% fresh shredded waste and 20% used animal bedding.

- Increasing the amount of UAB (used animal bedding) generally had positive influences on earthworm survival rate, reproductive rate and cocoon hatching rate and also on the nitrogen content of the vermicompost produced. However, the UAB addition (N inputs) altered the nitrogen cycle in the vermicomposting system. This changed the forms of nitrogen content in the vermicompost and resulted in a great amount of nitrogen loss (old shredded waste substituted with 20% used animal bedding). Vermicompost derived from fresh shredded waste substituted with 20% used animal bedding also had a relatively high total nitrogen content, but the nitrogen contained in it was more in the form of ammonia rather than nitrate.

- Applications of vermicompost had consistent growth promoting effects on Pak Choi, such as increasing leaf number, leaf area, plant dry weight and shoot and root length. These effects occurred in all growth periods and also affected plant structure.

- Although applications of vermicompost derived from domestic wastes (municipal organic waste and used animal bedding) had positive influences on plant growth, its low nitrogen content meant that a higher application rate may be needed to fulfil the nitrogen requirement of plants, compared with other nitrogen-rich vermicomposts. The study also found that combining vermicompost and chemical fertiliser was a good strategy to diminish the low nitrogen content of these domestic waste-generated vermicomposts and achieve maximum growth promotion effects.

- Vermicompost effects on plant growth can be influenced by factors such as medium type, the source of vermicompost, concentration of vermicompost, application method, processing method and chemical fertiliser addition. Interactions among each factor were observed in this study. Coir was found to have a good combinability with vermicompost, which is showing that it could be a promising medium for combining with vermicompost for use in horticultural production.

6.2 Recommendations for future research

This study investigated the feasibility of vermicomposting domestic waste (municipal organic waste and used animal bedding) in New Zealand and the effects of the vermicompost produced on plant growth. However, there were some problems questioned, and research in the following area may need to be covered in order to implement vermicomposting technology in practice.

- Correlations between earthworm morphology and earthworm health were observed. It is speculated that this (allocating morphology changes) could be a possible method to investigate the nutrient requirements of earthworms and to assess the toxicity of certain substances to earthworms. Further research in this area is needed.

- Although the 80% fresh shredded waste with 20% used animal bedding appeared to be the ideal mixture for use as a raw material for vermicomposting, the sanitisation status of the waste generated vermicompost is unknown, since there is no thermophilic stage. Additional tests may be needed to assess whether this factor will create problems if such vermicomposts used in horticultural production.

- Domestic waste generated vermicomposts have a low nitrogen content. This had effects on Pak Choi growth, especially when the medium did not have another nutrient (chemical fertiliser) supply. However, previous studies have found that some amendments added to the feeding materials may improve the nutritional quality of vermicompost. Further studies could test whether these amendments can improve the nitrogen content of vermicompost if they are mixed with these wastes.

- The results of the pot experiments indicated a complex interaction between medium type, source of vermicompost, concentration of vermicompost, application method, processing method and chemical fertiliser addition. The possible mechanisms behind these interactions may associate with the changes in the physical, chemical and biological properties of the growth medium. Nevertheless, it is still not know how these changes interact with each other to result in the growth promoting effects observed. Further studies are required in this area.

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